The indenoindoles: a novel family of antioxidants

Graupner, Paul R.

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The indenoindoles:

A novel family of antioxidants

submitted by PAUL R. GRAUPNER

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ProQuest LLC
789 East Eisenhower Parkway
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"All men have secrets and here is mine, so let it be known..."

(Morrissey, from "What difference does it make")

To my parents

Acknowledgements

I would like to thank all the people who have made this work possible, and who have made my stay at Bath an enjoyable one. Special thanks are due to my supervisor Dr. Malcolm Sainsbury for useful discussion and encouragement over the three years, and for all my colleagues and friends at Bath for much help and many discussions late into the evenings.

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Finally, I would like to thank my close family and friends for their love and support (and occasional kicks up the backside when required!) throughout my education.

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EXPERIMENTAL

MODELS OF THE *VINCA* ALKALOIDS

Abstract

The discovery and investigation of a novel family of antioxidants based on the tetracylic indeno[1,2-*b*]indole carbon skeleton is described. The family divides into two groups, the dihydro- derivatives bearing an indole group, and the tetrahydro- derivatives, where the pyrrole ring is partially reduced as an indoline. The most active compound in the indole series is *5,10-dihydro-10,10-dimethylindeno[1,2-*b*]indole* where dialkyl substitution at C-10 provides steric isolation of the pyrrole aromatic sextet, and which protects the radical cation formed on one electron oxidation. In the indoline series the best compound is *4b,5,9b,10-tetrahydro-6,8-dimethylindeno[1,2-*b*]indole* where the radical formed is protected from reaction through the aromatic ring.

Evidence is given that the compounds inhibit hydrogen abstraction as well as one electron oxidation, and that during this process, the tetrahydro compounds dehydrogenate to their respective dihydro derivatives.

Evidence from electrochemical investigation reveals that some of the substrates, especially *4b,5,9b,10-tetrahydro-8-methoxy-5-methylindeno[1,2-*b*]indole*, form very persistant radical cations on one electron oxidation, revealing very good redox couples in cyclic voltammetry.

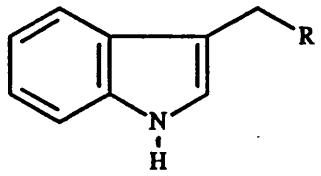
Additionally, a proposal is put forward for the mode of action of the anti-cancer *Vinca* alkaloids vinblastine, and vincristine, and some approaches to the synthesis of models of the alkaloids are described.

Abbreviations.

Ac	Acetyl
AIBN	Azo-*bis*-(*iso*-butyro)nitrile
Ar	Aryl
n-Bu	"Normal" butyl
t-Bu	Tertiary butyl
BP	Benzo[*a*]pyrene
CI	Chemical ionisation
CPI	1,2,3,4-Tetrahydrocyclopent[*b*]indole
COSY	Correlation spectroscopy
CV	Cyclic voltammetry
δ_H	Proton chemical shift in parts per million
δ_C	Carbon chemical shift in parts per million
DCM	Dichloromethane
DHII	5,10-Dihydroindeno[1,2-*b*]indole
DMSO	Dimethyl sulphoxide
EI	Electron impact
esr	Electron spin resonance
Et	Ethyl
eV	Electron volt
Fig.	Figure
g.l.c.	Gas-liquid chromatography
HHC	1,2,3,4,4a,9b-Hexahydrocarbazole
HHCPI	1,2,3,3a,4,8b-Hexahydrocyclopent[*b*]indole
hr	Hour
I-3-C	Indole-3-carbinol

i.r.	Infra red
lit.	Literature
J	Coupling constant
Me	Methyl
m.p.	Melting point
m/z	Mass:charge ratio
m.s.	Mass spectrometry
nmr	Nuclear magnetic resonance
Ph	Phenyl group
^{i}Pr	*iso*-propyl
R	Alkyl
R_F	Retention factor
SAR	Structure/activity relationship
TBATFB	Tetrabutylammonium tetrafloroborate
THC	1,2,3,4-Tetrahydrocarbazole
THF	Tetrahydrofuran
THII	4b,5,9b,10-Tetrahydroindeno[1,2-*b*]indole
t.l.c.	Thin layer chromatography
TMEDA	*N*,*N*,*N'*,*N'*-Tetramethylethylenediamine
TMS	Tetramethylsilane
tosyl	*p*-toluenesolphonyl
u.v.	Ultra violet

In 1975, three indoles (1–3), which occur naturally in vegetables of the *Brassica* family (for example, brussel sprouts and cabbages), were shown to inhibit the formation of cancer of the forestomach in mice exposed to benzo[*a*]pyrene (BP).[1, 2]

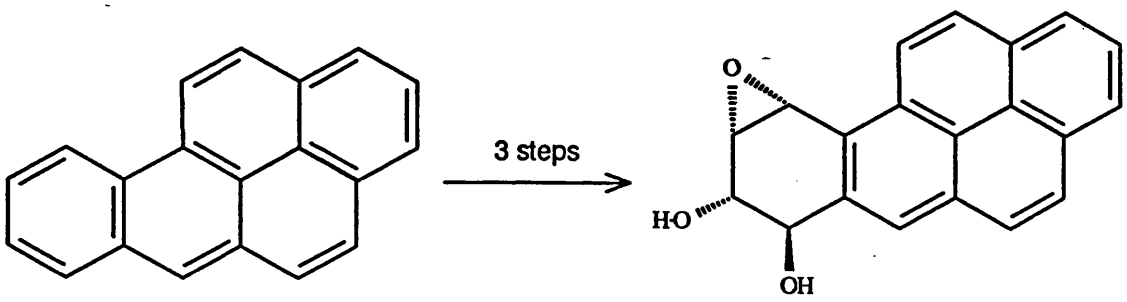


(1) R = -OH

(2) R = -CN

(3) R = 3-indolyl

BP (4) is itself not a carcinogen, but it is known to be activated *in vivo* to the diolepoxide (5), (scheme 1:1), which is the true carcinogen. This oxidative process, is mediated in the liver by cytochrome P450.[3, 4]

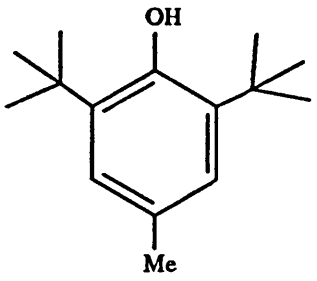


(4) (5)

Scheme 1:1

Certain phenols such as 2,6-di-*tert*-butyl-4-methylphenol (BHT) (6), and 3-*tert*-butyl-4-hydroxyanisole (BHA) (7), are also known to inhibit BP carcinogenesis,[5, 6] and since these compounds are also known to act as antioxidants (for instance as food additives E321 and E322 respectively), it seems likely that these two processes are linked.

During oxidation, the phenols are converted to their respective phenoxy radicals



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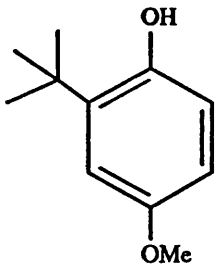
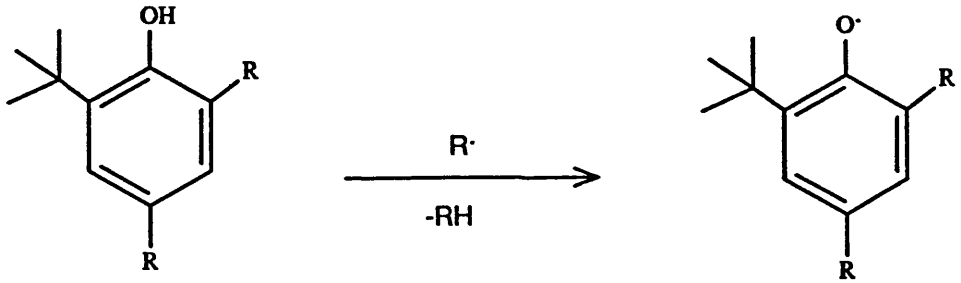


(7)

(scheme 1:2), which are stabilised by steric isolation of the free radical by the *tert*–butyl groups. Further stabilisation in the case of BHA (7) is given by conjugation of the radical with the *para*–methoxy substituent, whereas in the example of BHT (6), the *para*–methyl group delocalises the unpaired electron through hyperconjugation, and prevents further reaction through this position.

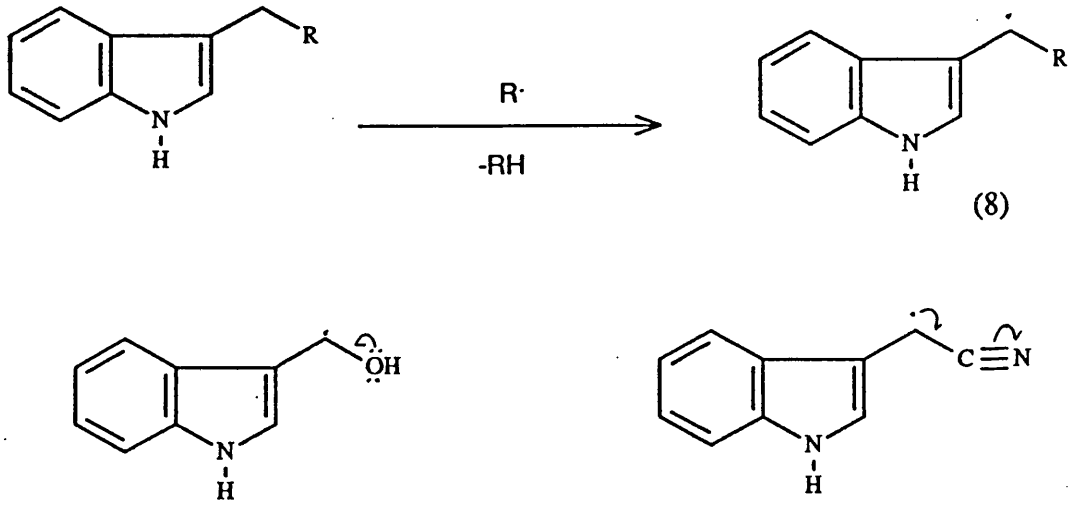


Scheme 1:2

In this way, the phenols effectively terminate chain reactions by scavenging reactive and harmful free radicals, to form stable phenoxy radicals.

At first, Sainsbury argued that the three indoles (1–3) acted in a similar fashion to these "suicide substrates", forming stable radicals of the type (8) – these being stabilised by conjugation of the unpaired electron at the methylene bridge with the π–system of the pyrrole ring, and also with the electrons of the adjacent substituent group. This seemed to be the only way to explain why electron withdrawing and electron donating substituents attached to the methylene gave compounds offering similar biological effects (scheme 1:3).

In order to optimise this property, certain obvious modifications to the indoles



Scheme 1:3

might be made, including the synthesis of 3-benzylindoles, where delocalisation of any free radical over the aryl group would certainly improve the stability of the corresponding radical.

This work was commenced by my predecessor Dr. Iain Hogan, but a major problem was soon encountered: that of finding a non-commercial collaboration in which the synthesised compounds might be screened as inhibitors of carcinogenesis - an expensive business! Outside of major pharmaceutical companies, such collaborations are hard to come by as most pharmacologists have strictly defined avenues of research determined by specific funding interests.

However, Professor H. G. Shertzer at the University of Cincinnatti had shown that the indoles (1-3) inhibit the DNA binding of *N*-nitrosodimethylamine, which presumably is activated by oxidation. Secondly. Shertzer had demonstrated that the indoles inhibit lipid peroxidation.[7] An assaying procedure of this second property is relatively easily achieved, and so contact was made with Shertzer's group in the successful hope that he would agree to assay our synthetic compounds as antioxidants.

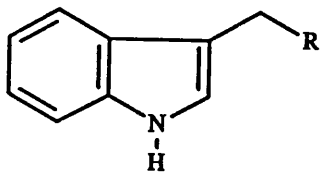
The first compounds to be synthesised, were the 3-benzyl- or 3-pyridylmethylindoles (9-13), designed to enhance the stabilising effect of the group

appended to the methylene function. Three different effects were investigated. the appended group containing:

i) an electron donating group (compound 10)

ii) an electron deficient group (11–12)

iii) a bulky group (13).



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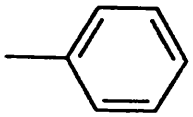

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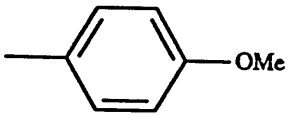
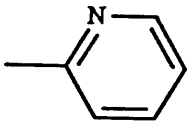
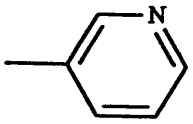


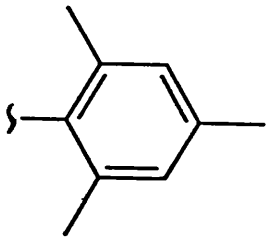
(11) R =



(12) R =



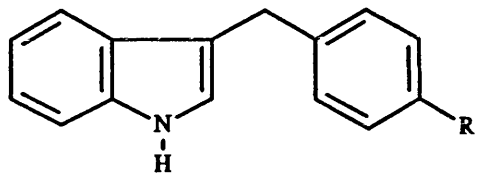
(13) R =



Skatole (3–methylindole) was also obtained as a control to investigate the necessity of a stabilising group at all.

In a phospholipid oxidation assay (the biological tests will be described in greater detail in chapter 4), it was found that 3–(2,4,6–trimethylbenzyl)indole (13) was the best antioxidant, rating higher than any of the naturally occurring indoles (see table 1:1). Also compounds with an electron donating substituent on the benzyl group, were better antioxidants than those with electron withdrawing substituents, and so indoles contain–

ing a benzyl group with two different electron donating groups were prepared and assayed (compounds 14–15).



(14) R = OH

(15) R = NMe_2

Of these, 3-(4-*N*,*N*-dimethylaminobenzyl)indole (15) proved to be the best indole compound, although in one assay this still rated lower than BHT (6).

To test further the hypothesis that the methylene position was important, three additional compounds were synthesised, making the following changes:

i) the methylene group was replaced with a carbonyl group (compound 16)

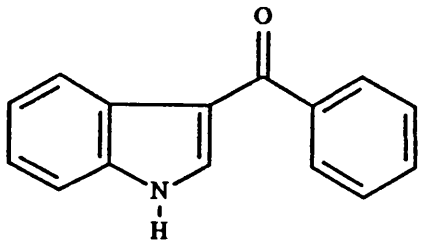
ii) two methyl groups were incorporated into the bridge effectively bocking radical formation at this site (17)

iii) the bridge was homologated into an ethyl unit therby isolating the aryl and heterocyclic π-systems (18).

The surprising result from this was that 3-(2-methyl-2-phenylethyl)indole (17), was a better antioxidant than 3-benzylindole (8).

Attention was then turned to the role of the nitrogen atom on the pyrrole ring. *N*-Methyl derivatives of three previously tested compounds [3-benzyl- (8), 3-(4-methoxybenzyl)- (9), and 3-(2-methyl-2-phenylethyl)indole (17)] were prepared. Only a slight variation in activity of these was noted (see table 1:1), except in the case of 1-methyl-3-(2-methyl-2-phenylethyl)indole (19), where all antioxidative properties were destroyed.

The final compound to be synthesised in this initial study, was designed to investi-



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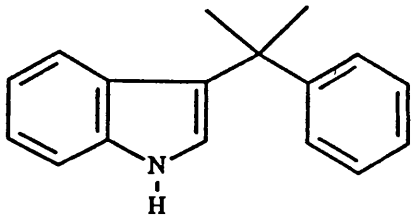

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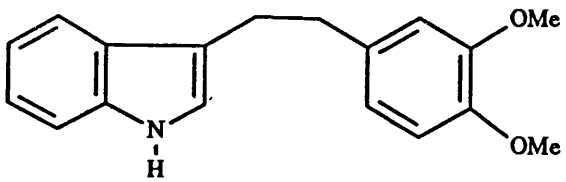
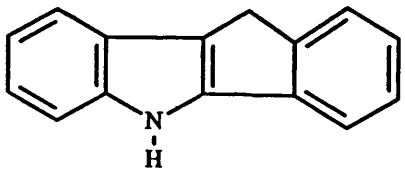


(18)

gate the effect of ring fusion. 5,10–Dihydroindeno[1,2–*b*]indole (20) was prepared and found to be the best compound tested so far, exhibiting better antioxidative properties even than BHT and α–tocopherol (vitamin E), two standards used in the assay [synthetic α–tocopherol, like BHT and BHA is used as a food additive (E307)].



(20)

Obviously this tetracyclic ring system was worthy of further study to determine the mode of its action. It is this study which forms the major part of the work described in this thesis.

The results in table 1:1 show the action of the compounds mentioned above in two oxidative systems:

i) phospholipid oxidation initiated by ferrous/ascorbic acid in a phosphate

buffer (pH 7.4)

ii) non-aqueous oxidation of phospholipid in chlorobenzene, initiated by azo-*bis*-isobutyronitrile (AIBN).

Each result is given by the amount of indole compound required to inhibit the respective oxidation by 50% (a more detailed description of the first of these procedures is given in chapter 4).

Table 1:1

Biological data of 3-methyleneindole derivatives

Compound	50% I (Fe) μm	50% I (AIBN) μm
5,10-dihydroindeno[1,2-*b*]indole (20)	<4	14
BHT (6)	1.2	18
3-(4-dimethylaminobenzyl)indole (15)	1.5	16
α-tocopherol	10	40
3-(2,4,6-trimethylbenzyl)indole (13)	11	100
3-(4-hydroxybenzyl)indole (14)	12	13
3-[2-(3,4-dimethoxyphenyl)ethyl]indole (18)	12	115
3-benzyl-1-methylindole	13	500
3,3'-diindolylmethane (3)	15	64
3-(1-methyl-1-phenylethyl)indole) (17)	18	80
3-(4-methoxybenzyl)indole (10)	24	300
3-(4-methoxybenzyl)-1-methylindole	31	400
3-benzylindole (9)	36	185
indole-3-carbinol (1)	300	120
3-(2-pyridinylmethyl)indole (11)	325	200
3-(3-pyridinylmethyl)indole (12)	500	500
indole	800	1250
3-(4-methoxybenzoyl)indole (16)	>>250	>>250
indole-3-acetonitrile (2)	>1250	1250
1-methyl-3-(2-methyl-2-phenylethyl)indole (19)	>1250	>1250
skatole	–	–

References to chapter 1

1. L. W. Wattenburg, *Cancer Res.*, 1975, **35**. 3326.

2. W. B. Loub and L. W. Wattenburg, *Cancer Res.*, 1978, **38**, 1410.

3. I. T. Hogan, *Ph.D. Thesis (University of Bath)*, 1985.

4. M. R. Osbourne and N. T. Crosby, *Benzopyrenes: Cambridge Monographs On Cancer Research*, Cambridge (1987).

5. L. W. Wattenburg, *J. Natl. Cancer Inst.*, 1972, **48**, 1425.

6. L. W. Wattenburg, J. B. Coccia, and L. K. T. Lam, *Cancer Res.*, 1980 , **40**, 2820.

7. H. G. Shertzer, M. L. Berger, and M. W. Tabor, *Biochem. Pharmacol.*, 1988, **37**, 333.

2:1 Autoxidation and Antioxidants

Autoxidation is the process by which chemical compounds react with atmospheric oxygen. With certain functionality, this oxidation is particularly facile, leading to breakdown of the substrate, giving peroxides (see section 2:1:2). Particularly sensitive to atmospheric oxygen, are unsaturated lipids such as those found in living tissue. Yet even though these lipids are present in all living cells, quenched in oxygen, and in the presence of of transition metals which are powerful initiators of oxidation, the material does not degrade at any appreciable rate. This is because living tissue also contains natural antioxidants which are as ubiquitous as the unsaturated lipids themselves.

In healthy tissue, these antioxidants act by scavenging oxidising species therefore preventing damage to lipids and they are constantly recycled. After death, the recycling process stops, and once the store of antioxidants has been depleted, the body starts to absorb oxygen at a rate much greater than at any time during life (for a monograph on the mechanisms of natural antioxidants in the human body, the reader is directed to the article by Dormandy in "The Lancet"[1]).

Autoxidation of organic material also takes place in many commercial products, and also in foods. Indeed, the prevention of rancidification of foodstuffs, particularly butter and cooking oils, which contain unsaturated fats (lipids) is vitally important, and many synthetic antioxidants have been developed.

Food antioxidants bear certain characteristics as listed by Porter:[2] a good antioxidant must be effective as a hydrogen or electron donor; the resulting radical must be sufficiently stabilised so that the electron density of the unpaired electron is not localised, and thus reactive towards chain transfer, similarly the radical should be sterically hindered for maximum effect; the original species

must be stable towards autoxidation itself; and the compound must be sufficiently lipid soluble so that it is concentrated in areas in which it is most effective. Two types of compounds exhibit most of the above characteristics, these being arylamino and arylhydroxy compounds. The former are usually disregarded, as radicals centred on nitrogen tend to form nitroso compounds which are often toxic. Even so one or two have found some use as antioxidants (*e.g.* diphenylamine, used to prevent discolouration of fruit).

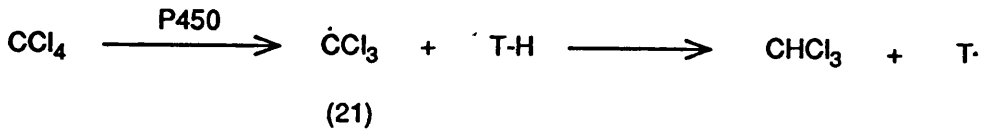
The compounds which have attracted the most interest as commercial antioxidants are mono– or polyhydric phenols of which the three commercial antioxidants mentioned in chapter 1 BHT, BHA and α–tocopherol are good examples.[3,4]

2:1:1 *Antioxidants and Carcinogenesis*

Antioxidants such as BHA and BHT have been shown to prevent the activation of BP into its carcinogenic derivatives *in vivo*, but exactly how this is achieved is still a matter of debate. Wattenberg[5] favours the stimulation of the oxidative system of the host so that overoxidation of the potential carcinogen occurs leading to easily excreted non–toxic metabolites. This problem is complex, however, and it is known that large doses of BHA and BHT can give rise to the formation of carcinoma.[6] Because of this, and the paucity of knowledge about the effects of existing antioxidants, there is a need for novel non–toxic antioxidants.

One procarcinogen which is known to act as a free radical agent is carbon tetrachloride.[7] The active species is the trichloromethyl radical (21) released by the action of cytochrome P450, which attacks polyene fatty acids present in the adjacent lipoprotein membrane, and then destroys P450 itself.[8] Hepatoma are the usual pathological result and such damage is easily detected. The tri–chloromethyl radical is itself easily detected by spin–trapping techniques *e.g.*

with phenyl-*tert*-butylnitrone, and the adduct studied by esr.[9] The whole procedure can be used as a test system for formation of the radical by P450 contained in rat liver microsomes. As the radical is produced a triplet signal is noted in the esr spectrum. If α-tocopherol (T-H) is present, the intensity of this signal is increased demonstrating that the P450 is "protected" and metabolises more molecules of carbon tetrachloride. It is not known in this case whether the radicals captured by the spin trap are trichloromethyl radicals, or stable radicals formed from the reaction of $\dot{C}Cl_3$ with α-tocopherol (scheme 2:1).



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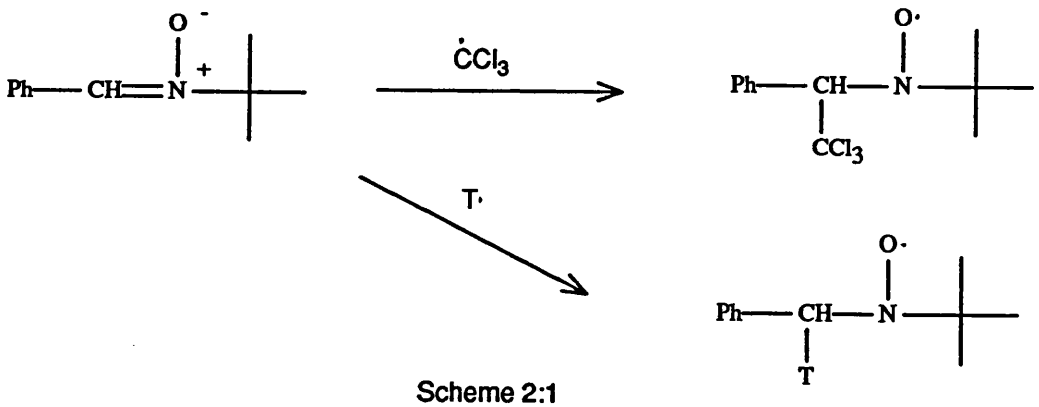


Scheme 2:1

Potential antioxidants which protect lipid membranes must themselves be lipophilic, for example the long phytyl chain of α-tocopherol is an ideal handle with which the the molecule can be "locked" into the vulnerable membrane (for further details on this aspect of the mode of action of α-tocopherol, see section 3:4).

2:1:2 *Autoxidation of Organic Compounds*

Compounds which undergo autoxidation include tertiary alkanes, alkyl eth-

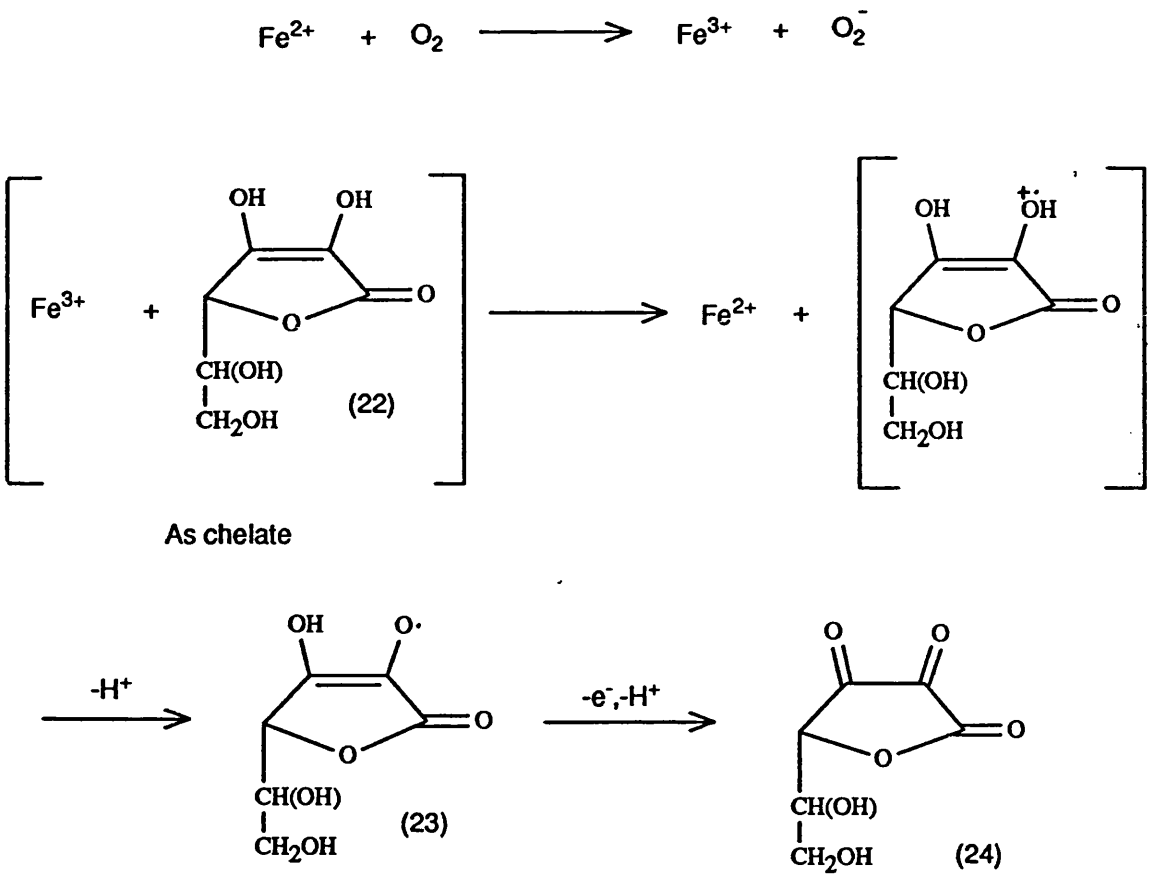
ers, aldehydes, and allyl derivatives. Initiation of the process normally requires hydrogen abstraction giving a radical which reacts with oxygen to form a peroxy radical which then serves to propagate a chain reaction. Our initial attempt to assay the compounds synthesised in this study, was through monitoring their ability to inhibit lipid peroxidation initiated by iron in the presence of ascorbic acid.

Lipid peroxidation is a very complicated process the chemistry of which has been reviewed extensively.[10] In iron activated systems, it appears that the peroxidation is initiated by an iron(III)–superoxide complex,[11] this species being produced by a redox reaction between iron(II) and oxygen itself. The concentration of the oxidised iron(III) is kept low *in vivo*, and in the assaying system, by the presence of ascorbic acid (22), which serves to reduce it back to iron(II). Indeed it has been demonstrated that an excess of ascorbic acid will itself inhibit the peroxidation process,[12] presumably by removing all of the oxidised iron before the process is initiated (scheme 2:2).

Importantly, the intermediate ascorbyl radical (23) is a relatively inert species, and as such is non-toxic. This is an essential factor, and ascorbic acid serves to protect mammals against lipid damage. The ascorbyl radical – which has been detected in lyophilised tissue[13] – loses an electron and a proton, on further oxidation, to form dehydroascorbic acid (24). Our indolic antioxidants may act in a similar manner to ascorbic acid and become oxidised to a stable radical/radical cation by the action of Fe(III) ions, or they may serve as a trap for superoxide species.

2:2 Indoles

All the compounds evaluated by the author as potential inhibitors of lipid peroxidation were tetracyclic monomers bearing an indole nucleus. Since we



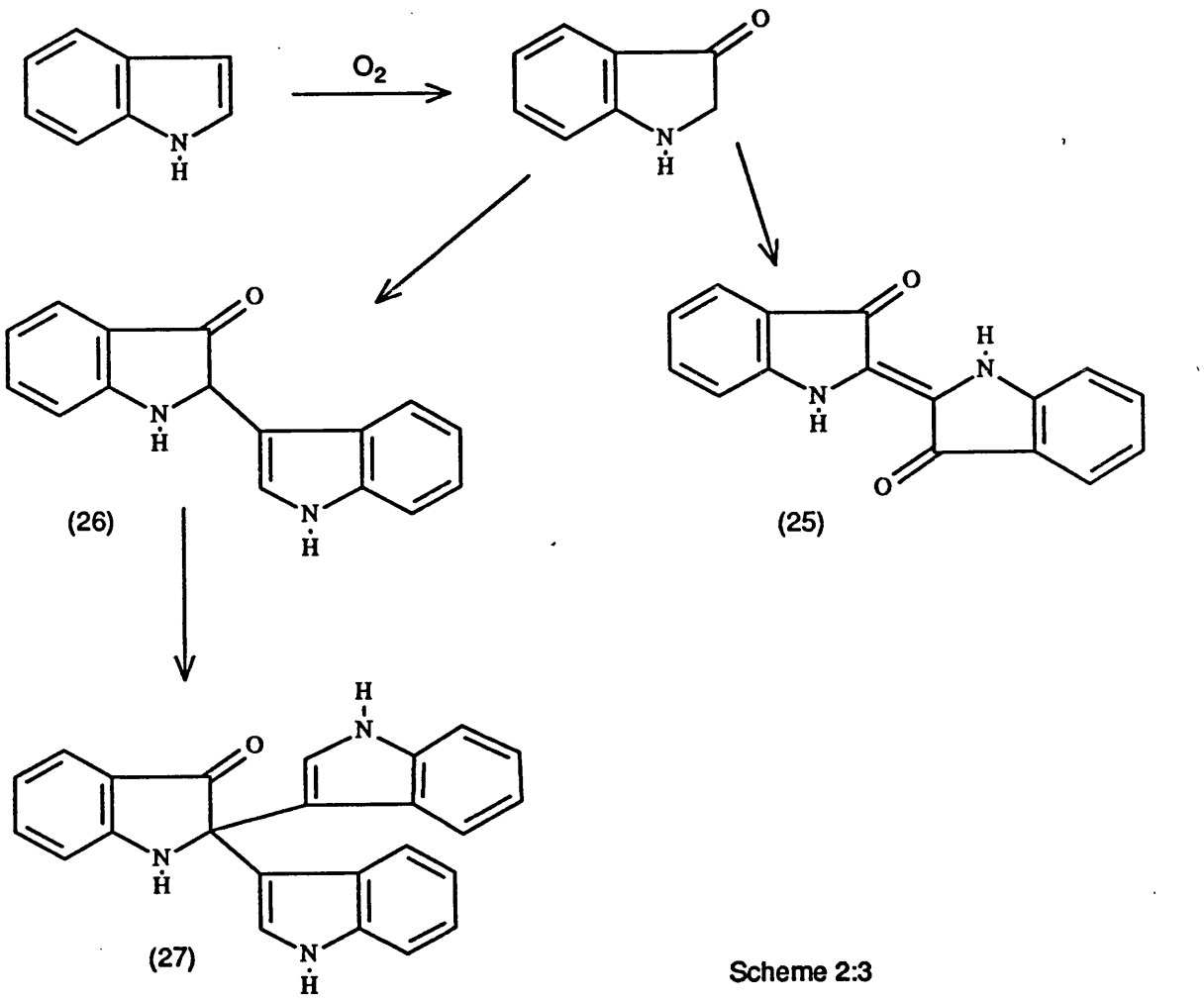
Scheme 2:2

were much interested in the fate of these molecules, during and after oxidation, it is appropriate at this point to survey what is known concerning the oxidative behaviour of indoles and related structures.

2:2:1 *Autoxidation of Indoles*

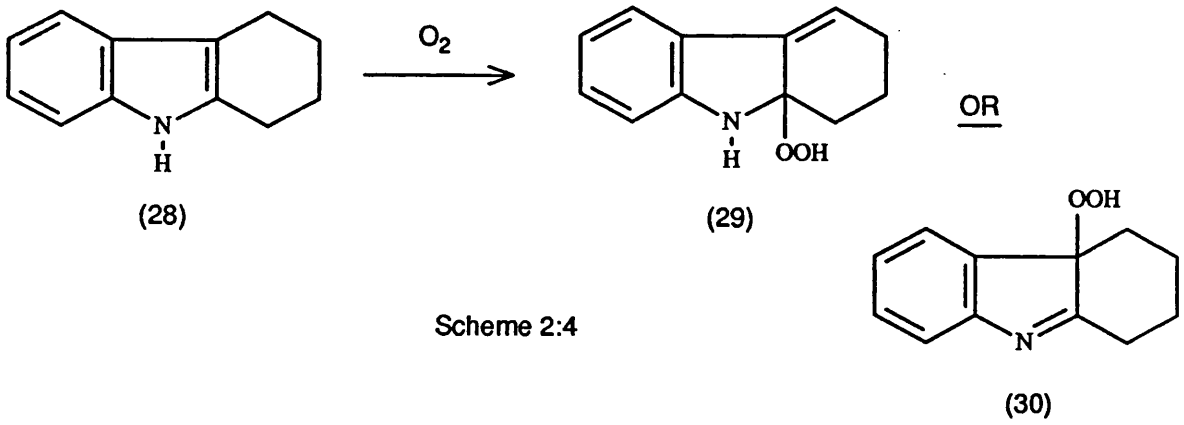
The main products formed from the oxidation of indole were determined in the early part of the century to be indoxyl, the dehydrodimer (25), the related structure (26), and 2,2 –diindolylindoxyl (27) (scheme 2:3).

Work in the 1950's by Beer *et al.* and Witkop *et al.* provides the basis for our understanding of the mechanism by which these and other products form. In the case of 1,2,3,4–tetrahydrocarbazole (THC, 28) for example, the initial product is a hydroperoxide, thought initially to be either the 2– or 3– substituted



Scheme 2:3

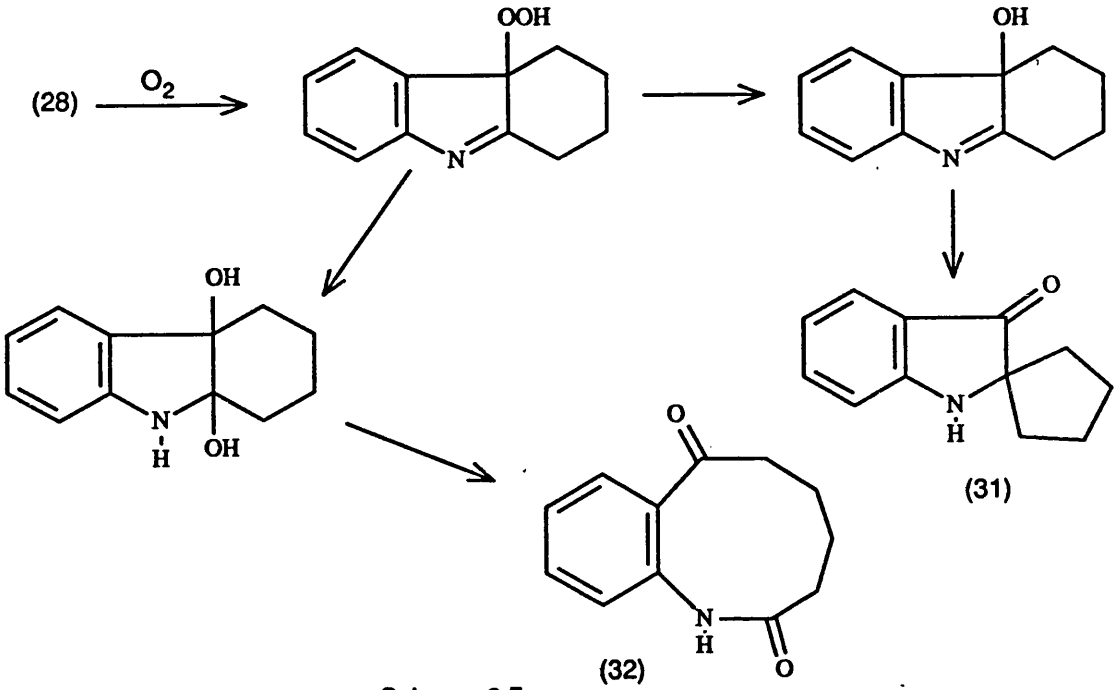
derivatives (29) or (30)[14] (scheme 2:4).



Scheme 2:4

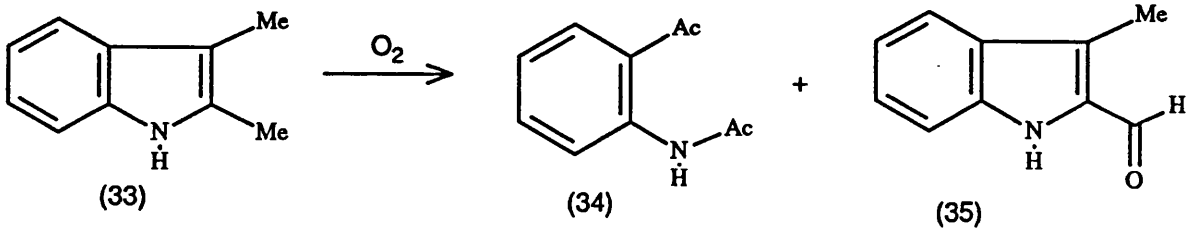
The correct structure is the latter[15] and this may breakdown to the hydroxyindolenine, and thence to the indoxyl (31).[16] Alternatively the hydroperoxide may

undergo another rearrangement to give the ketoamide (32)[17] (scheme 2:5).



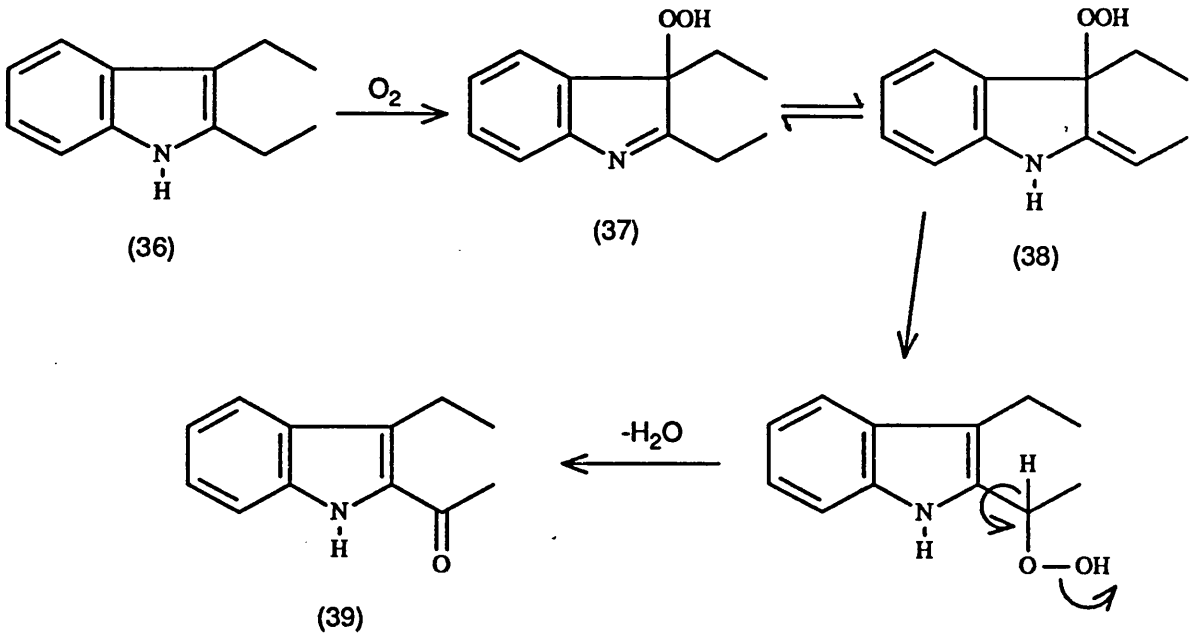
Scheme 2:5

The oxidation of simpler 2,3–disubstituted indoles has also been well researched. The main product from the autoxidation of 2,3–dimethylindole (33) is 2–acetylacetanilide (34), but a small amount of 2–formyl–3–methylindole (35) has also been identified[18] (scheme 2:6).



Scheme 2:6

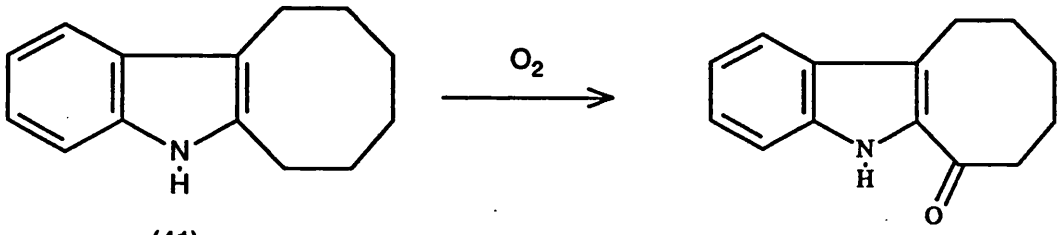
Leete has obtained a similar product in greater yield on the autoxidation of 2,3–diethylindole (36).[19] The mechanism depends on the equilibrium between the indolenine (37), and the enamine (38). Once formed, the latter rearranges and dehydrates to the 2–acetylindole (39), (scheme 2:7).



(36) (37) (38) (39)

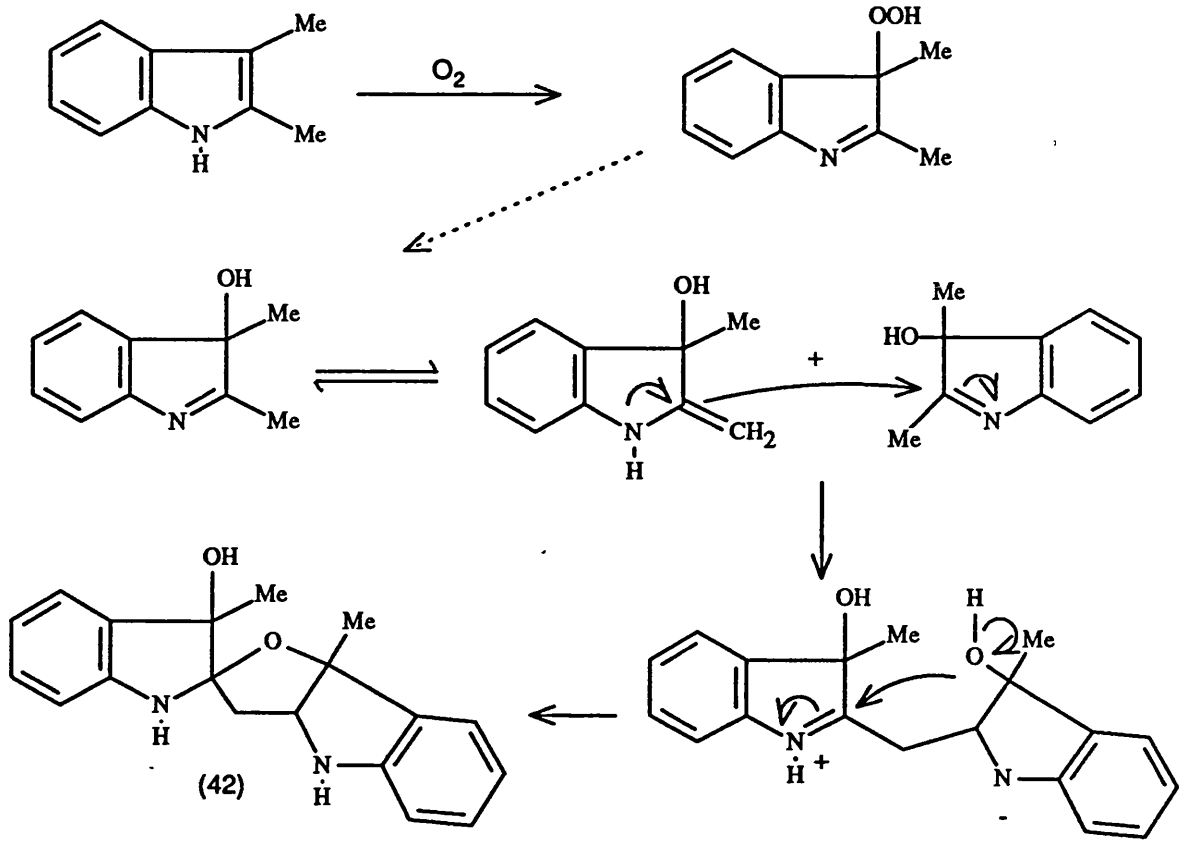
Scheme 2:7

This mechanism also accounts for the formation of the ketone (40) obtained previously by Witkop *et al.* from the autoxidation of cyclooct[*b*]indole (41)[20] (scheme 2:8).



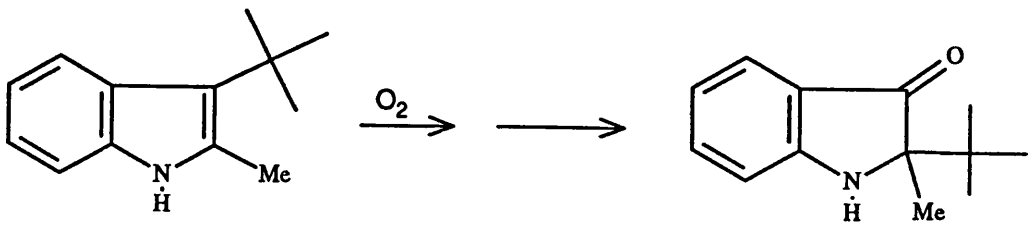
(41) Scheme 2:8 (40)

The indolenine/enamine equilibrium is implicated as the source of another autoxidation product obtained more recently from 2,3–dimethylindole (this compound autoxidises on recrystallisation from hexane). The spirocyclic tetrahydrofuran (42) was isolated and characterised by Berti *et al.*[21] and later confirmed by McLean;[22] the mechanism proposed invokes the dimerisation of one molecule of each tautomer of the indolenine (scheme 2:9).



Scheme 2:9

This dimerisation is prevented if a bulky group is present at C–3. Interestingly on autoxidation, the tertiary butyl group in 3–*tert*–butyl–2–methylindole (43) migrates [23] to give the 2,2–disubstituted indoxyl derivative (44), (scheme 2:10).



(43) (44)

Scheme 2:10

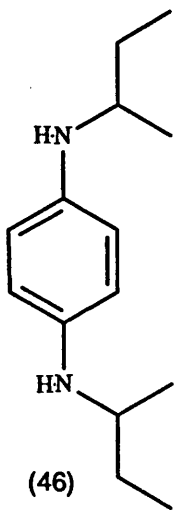
2:2:2 *Indoles as antioxidants*

There are few indoles which are effective antioxidants, and these are best classed along with amines. As we have mentioned, amines tend to be disregarded as food antioxidants however, due to the fear that they may be oxidised to form *N*–nitroso compounds, which are often toxic. Even so, some amines make very good antioxidants and may be useful in the protection of commercial products such as rubber or plastics.

Scott has summarised the structural requirements essential for amines to be effective antioxidants as follows:[3] a) there should be effective delocalisation for the unpaired electron formally resident on the nitrogen atom; b) there should be high electron density on the nitrogen, thus facilitating electron transfer from the atom to the oxidising species; c) there should be sufficient steric protection of the nitrogen to prevent chain transfer from the nitrogen atom. Whereas in phenols, this last property is covered by the addition of bulky groups *ortho* to the phenoxy substituent, in amines this is brought about by substitution on the nitrogen itself.

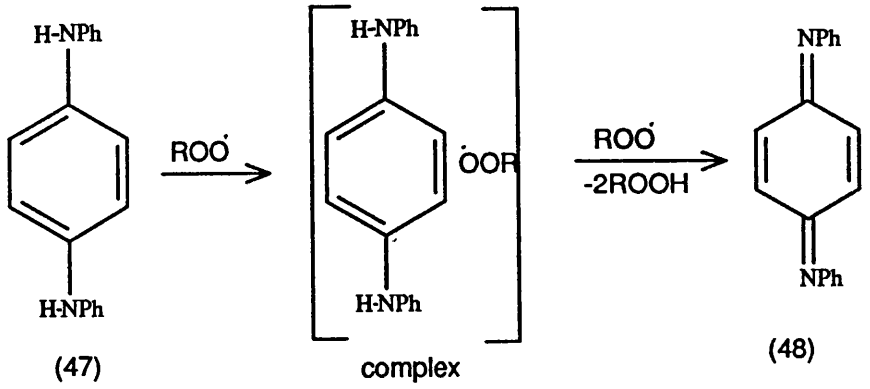
That amines can prevent autoxidation by radical transfer and not just *via* hydrogen abstraction, was demonstrated in 1956 by Pederson.[24] He showed that *N*,*N*,*N*',*N*'–tetramethyl–*p*–phenylenediamine (45) deactivates peroxy radicals, and is 36% as effective in this respect as *N*,*N*'–di–(*sec*–butyl)–*p*–phenylenediamine (46). As the former compound contains no labile hydrogen atoms, the mode of action must involve electron transfer from the nitrogen atom.

(45)



(46)

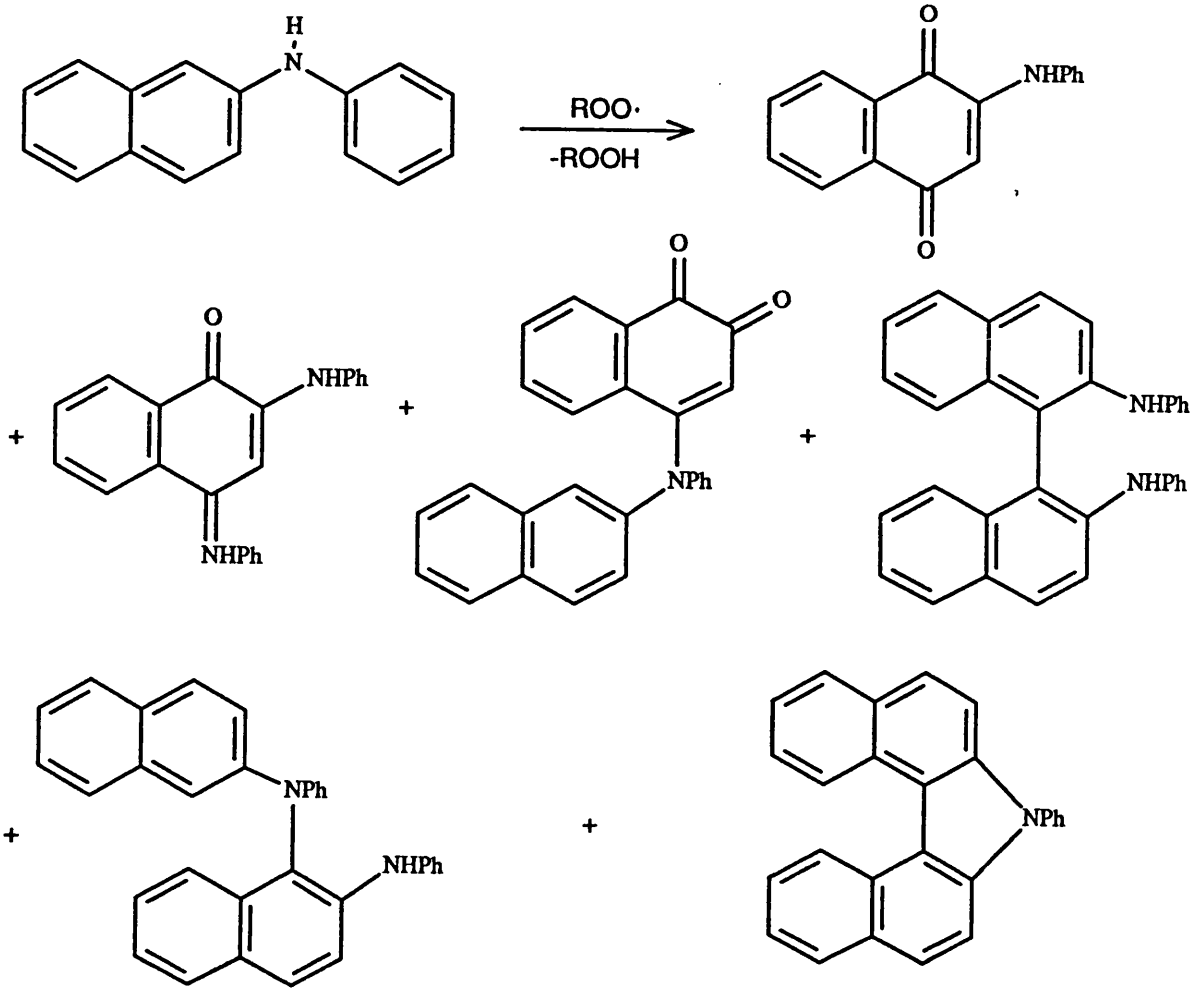
The product arising from the oxidative reaction of (47) is the diimide (48), and Boozer *et al.*[25] have shown that an initial complex forms between the diamine and a peroxy radical. This reacts with a second molecule of the radical to give the diimide (48) (scheme 2:11).



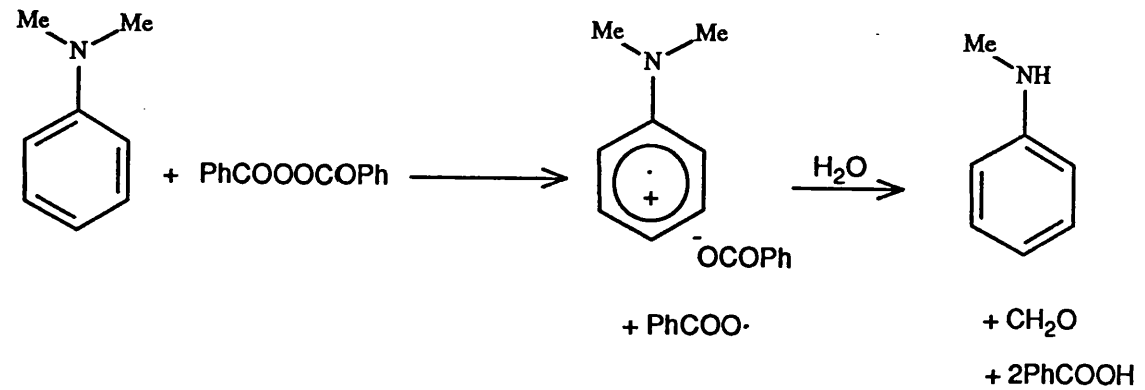
Scheme 2:11

In simpler arylated amines, the products from autoxidation are complex mixtures, and often the component compounds are themselves antioxidants and on prolonged oxidation, polymers are formed. For example Bowman *et al.*[26] identified six products from the reaction of *N*–phenyl–β–naphthylamine with peroxy radicals (scheme 2:12).

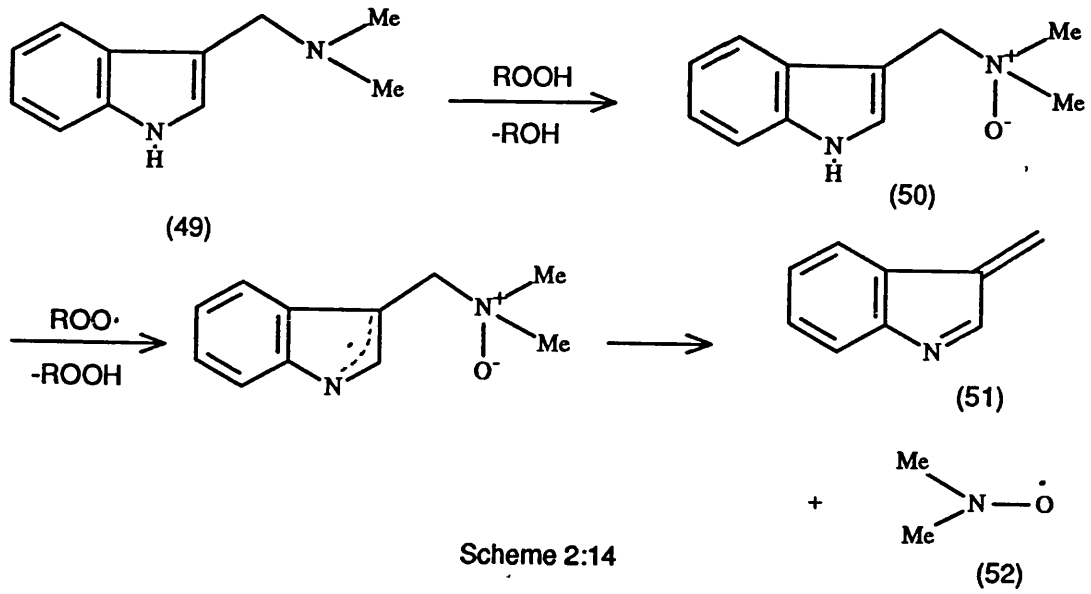
Tertiary aromatic amines may lose alkyl groups on oxidation. The reaction of dimethylaniline with benzoylperoxide, for example, yields methylaniline and formaldehyde is released (scheme 2:13). Gramine (49) is a good antioxidant,[27] its reactions with peroxides are very complicated, and lead to the formation of the *N*–oxide (50), which is an even better antioxidant than gramine itself. Reaction with further peroxy radicals leads to β–cleavage yielding the reactive 3–methyleneindolenine (51) (which readily polymerises or oxidises), and the dimethylnitroxyl radical (52), (scheme 2:14).



Scheme 2:12



Scheme 2:13



Scheme 2:14

2:2:3 *Electro-oxidation of Indoles*

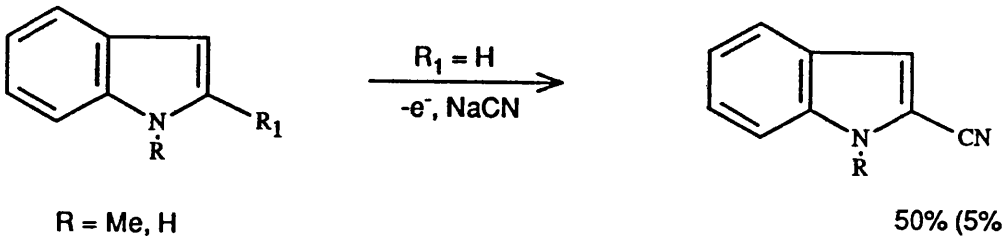
Little has been reported recently on the electro–oxidation of indole and its simple derivatives, and most information dates from the late 1970's. Oxidation leads to the loss of an electron to give the radical cation formally sited on the heterocyclic ring.

Yoshida[28] has shown that in the presence of cyanide ion, the radical ions from *N*–substituted indoles afford 2–cyanoindoles, but when this position is blocked attack occurs at C–3 (scheme 2:15).

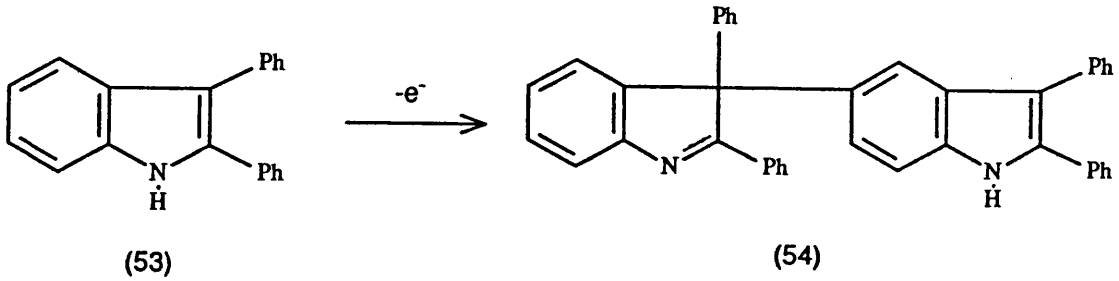
Yoshida does not comment on the mechanism of these reactions, but reports the results of molecular orbital calculations on the radical cation of *N*–methylindole[29] which show that there is greater charge distribution at C–2 rather than at C–3.

In the absence of nucleophiles, the radical cations from indoles dimerise, and Nelson *et al*[30] has shown that anodic oxidation of 2,3–diphenylindole (53) in acetonitrile containing triethylamine, gives the dehydrodimer (54), (scheme 2:16).

The authors suggest a mechanism for the dimerisation involving the

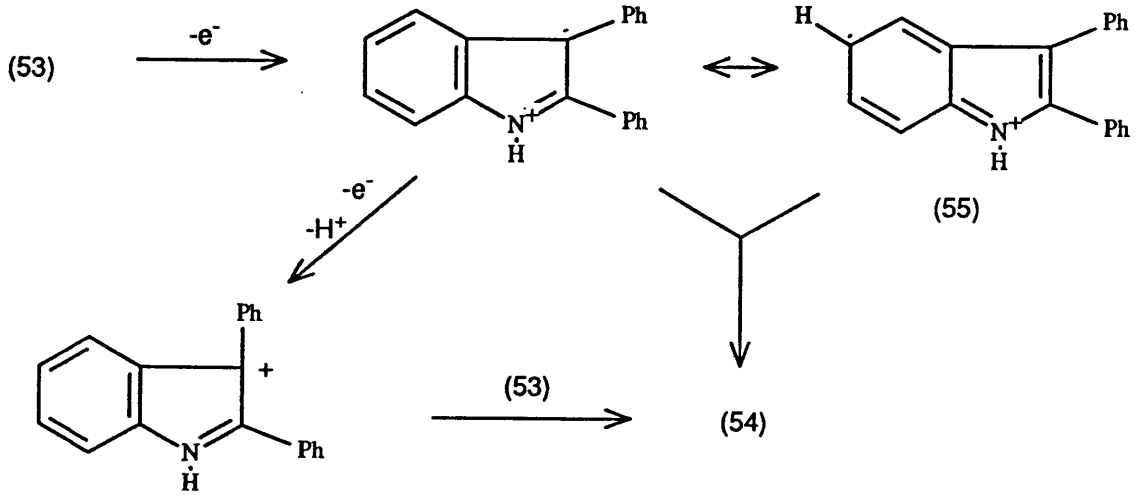


Scheme 2:15



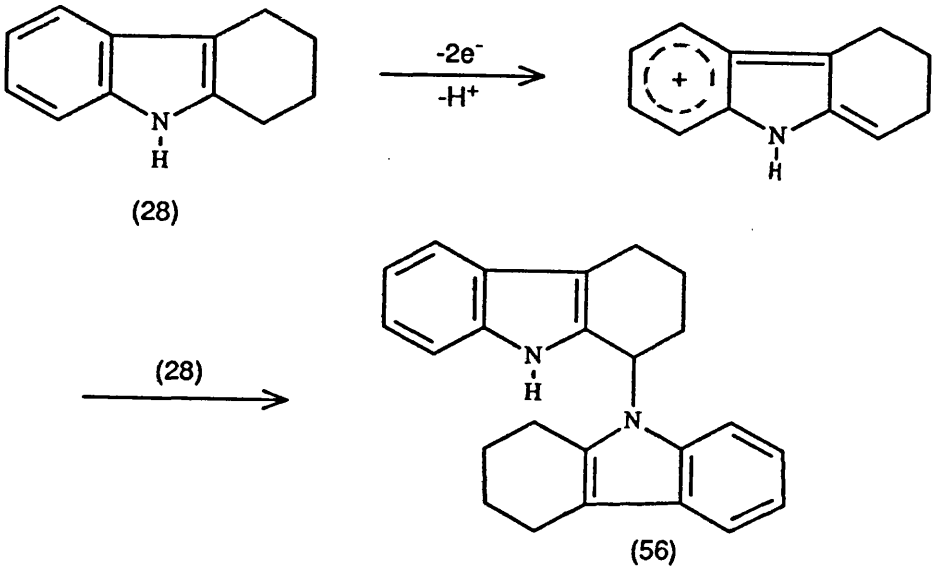
Scheme 2:16

coupling of two identical radical cations (55), however Bobbitt *et al.*[31] argue that the existence of a single unsymmetrical dimer from this reaction seems to suggest an ionic mechanism (scheme 2:17). There is no clear cut evidence to suggest either route as being the correct one.



Scheme 2:17

The formation of a dehydrodimer (56) has been observed during the electrolysis of 1,2,3,4–tetrahydrocarbazole (28). Here however, an ionic mechanism is suggested, involving a cationic intermediate, but without compelling evidence to support it[32] (scheme 2:18).

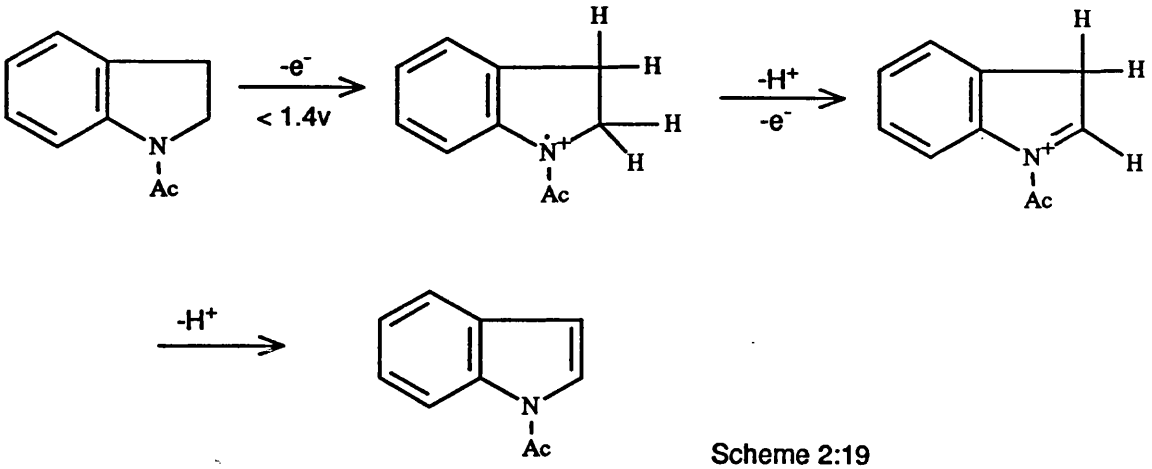


Scheme 2:18

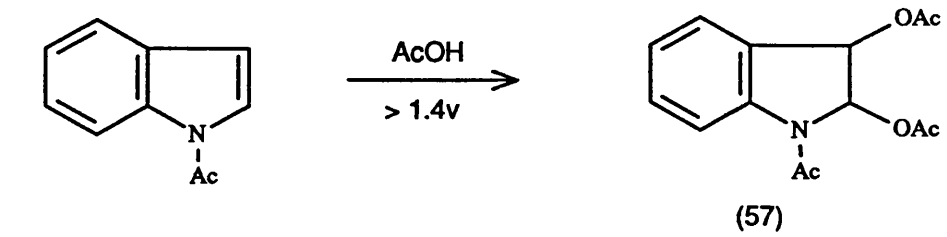
More complicated indole derivatives and indole alkaloids have also been the subject of electro–oxidation, but as these concern the interaction of additional pendant functionality, their description is beyond the scope of this work. This work has already been reviewed.[31]

There is little discussion of the electrochemical behaviour of reduced indoles in the literature, however when *N*–acetylindoline is oxidised in acetic acid/triethylamine, the ultimate product is 2,3–diacetoxy–1–acetylindoline (57). If the potential is controlled below 1.4 volts, dehydrogenation to *N*–acetylindole occurs, probably as indicated in scheme 2:19.[33]

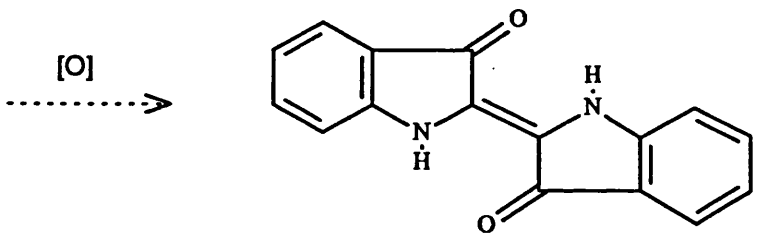
Should the potential be allowed to rise, the product *N*–acetylindole becomes oxidised and captures acetoxy anions from the electrolyte to yield (57). This compound, when exposed to water and air breaks down to 1,3–diacetylindoxyl, and then to indigo (scheme 2:20).



Scheme 2:19



(57)

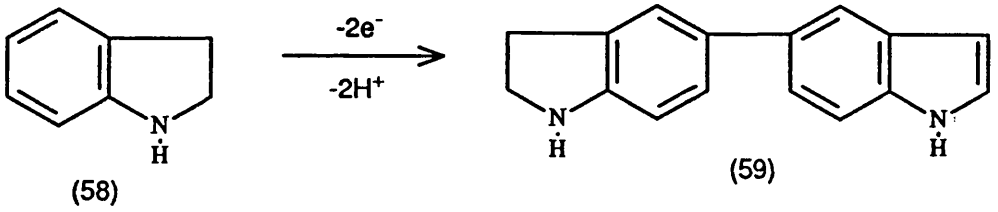


Scheme 2:20

Indoline (58), when oxidised, yields a green solid which is assumed to be the dehydrodimer (59) (scheme 2:21). This is a surprising result since indoline should be more easily oxidised than its *N*–acetyl derivative, and should also form indigo *via* the production of 3–acetylindoxyl. Interestingly, the dimer is unstable with a half–life of only a few hours decomposing to give a red amorphous solid.

2:3 Cyclic Voltammetry

When assessing electrochemical reactions, the technique of cyclic voltammetry is extremely valuable. The apparatus is simple: a ramp generator feeds current through a simple platinum bead electrode, the potential of which is



(58) (59)

Scheme 2:21

monitored by means of a reference electrode (usually a standard calomel); a counter electrode is also present; and the contents of the cell are not stirred. Often the bead electrode is initially maintained as the anode, but as a certain potential is reached, the voltage is reversed, and it becomes the cathode; hence the name of the process.

A simple example will serve to illustrate this concept. Compound A is placed in the cell, and at a certain potential (O_1) loses an electron to become a radical cation. Should this species be relatively stable, it survives near to, or adsorbed onto, the electrode surface, so that on the reverse sweep it may be reduced at potential R_1. This gives rise to a redox couple, since in practice O_1 and R_1 are usually of the same numerical value (fig 2:1).

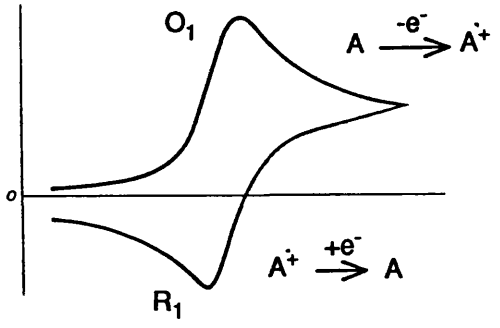


Figure 2:1

If, on the other hand, the radical cation $A^{\cdot +}$ enters into a fast chemical reaction, then the product B may give rise to to a further oxidation peak at O_2. On the reverse O_2 may have an associated reduction peak, but R_1 will be less obvious since little $A^{\cdot +}$ will be left unreacted. These events become obvious on the vol-

tammetric trace (I *versus* P) provided O_2 occurs at a greater potential than O_1 (fig 2:2a). However the product B may be more easily oxidised than A. In this case O_2 only occurs on the second and subsequent sweeps (fig 2:2b). Therefore it is important, when studying an electrochemical process, to scan a number of times to aid peak assignment.

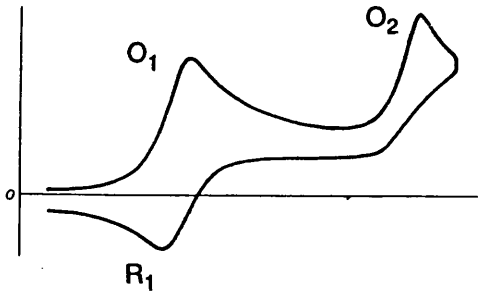


Figure 2:2a

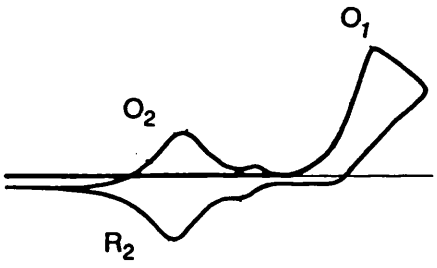


Figure 2:2b

There are obviously many variations on this theme, but cyclic voltammetry even in its most rudimentary form enables the chemist to determine at what potential to achieve ionisation, and whether the product of this initial ionisation is likely to react further if the potential of the electrolysis is raised. It may also be used to deduce the relative rates of the various processes involved because the scan rate of the experiment is in theory infinitely variable. We have therefore used cyclic voltammetry in our attempts to unravel the oxidation behaviour of our antioxidants (see chapter 4).

References to chapter 2

1. T. L. Dormandy, *The Lancet*, 647 (1978).

2. W. L. Porter, "Recent Trends in Food Applications of Antioxidants", in *Autoxidation in Food and Biological Systems*, ed. M. G. Simic and M. Karel, Plenum Press, New York (1980).

3. *Atmospheric Oxidation and Antioxidants*, Elsevier, Amsterdam (1965).

4. *Autoxidation in Food and Biological Systems*, Plenum Press, New York (1980).

5. L. W. Wattenberg, "Inhibition of Chemical Carcinogenesis by Antioxidants", in *Carcinogenesis Vol. 5: Modifiers of Chemical Carcinogenesis*, ed. Slaga, p. 85, Raven, New York (1980).

6. N. Ito, S. Fukushima, and H. Tsuda, *Crit. Rev. Toxicol.*, **15**, 109 (1985).

7. M. D. Reuber and E. L. Glover, *J. Natl. Cancer Inst.*, **38**, 891 (1967).

8. R. O. Recknagel, E. A. Glende Jr., and A. M. Hruszkewycz, *Free Radicals Biol.*, **3**, 97 (1977).

9. P. B. McCay, M. M. King, E. K. Lai, and J. L. Poyer, *Cancer Environ.*, 195 (1981).

10. N. A. Porter, *Methods Enzymol.*, **105**, 273 (1984).

11. J. R. Bucher, M. Tien, and S. D. Aust, *Biochem. Biophys. Res. Commun.*, **111**, 777 (1983).

12. H. G. Shertzer, M. L. Berger, and M. W. Tabor, *Biochem. Pharmacol.*, **37**, 333 (1988).

13. H. M. Swartz and N. J. F. Dodd, "The Role of Ascorbic Acid on Radical Reactions in vivo", in *Oxygen and Oxy Radicals in Chemistry and Biology*, ed. Rodgers and Powers, p. 161, Academic Press, New York (1981).

14. R. J. S. Beer, L. McGarth, and A. Robertson, *J. Chem. Soc.*, 2118 (1950).

15. R. J. S. Beer , L. McGarth, and A. Robertson, *J. Chem. Soc.*, 3283 (1950).

16. B. Witkop, *J. Am. Chem. Soc.*, **72**, 1428 (1950).

17. J. B. Patrick and B. Witkop, *J. Am. Chem. Soc.*, **72**, 633 (1950).

18. W. I. Taylor, *Proc. Chem. Soc.*, 247 (1962).

19. E. Leete, *J. Am. Chem. Soc.*, **83**, 3645 (1961).

20. B. Witkop, J. B. Patrick, and M. Rosemblum, *J. Am. Chem. Soc.*, **73**, 2641 (1951).

21. G. Berti, A. Da. Settimo, G. D. Colo, and E. Nannipieri, *J. Chem. Soc. (C)*, 2703 (1969).

22. S. McLean and G. I. Dmitrienko, *Can. J. Chem.*, **49**, 3642 (1971).

23. V. Dave and E. W. Warnhoff, *Can. J. Chem.*, **54**, 1015 (1976).

24. C. J. Pederson, *Ind. Eng. Chem.*, **48**, 1881 (1956).

25. C. E. Boozer, G. S. Hammond, C. E. Hamilton, and J. N. Sen, *J. Am. Chem. Soc.*, **77**, 3233 (1955).

26. D. F. Bowman, B. S. Middleton, and K. U. Ingold, *J. Org. Soc.*, **34**, 3456 (1969).

27. M. Schultz, H. Wegwart, G. Stampehl, and W. Riediger, *J. Polymer Sci.*, 329 (1976).

28. K. Yoshida, *J. Am. Chem. Soc.*, **99**, 6111 (1977).

29. K. Yoshida, *J. Am. Chem. Soc.*, **101**, 2116 (1979).

30. G. T. Cheek and R. F. Nelson, *J. Org. Chem.*, **43**, 1230 (1978).

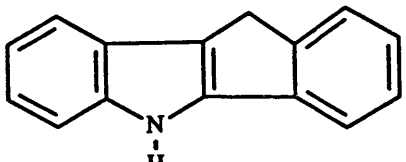
31. J. M. Bobbitt, C. L. Kulkarni, and J. P. Willis, *Heterocycles*, **15**, 495 (1981).

32. J. M. Bobbitt and J. P. Willis, *Heterocycles*, **6**, 899 (1977).

33. S. Torii, T. Yamanaka, and H. Tanaka, *J. Org. Chem.*, **43**, 2882 (1978).

3:1 Dihydroindeno[1,2-*b*]indoles

From our initial attempt to optimise the antioxidant activity of the 3–methyleneindole derivatives, it was demonstrated that 5,10–dihydroindeno[1,2–*b*]indole (DHII, 20) is a much better antioxidant than any of the three naturally occurring indoles.[1]



(20)

Indeed, DHII is a superior antioxidant than α–tocopherol (vitamin E), BHT, or BHA, three widely used commercial antioxidants.

Since the mode of action of DHII was unknown, the author's task was to investigate the behaviour of the compounds by:

i) the preparation of derivatives of DHII for an extended SAR study,

ii) carrying out an investigation of the chemical properties of DHII in order to see if oxidation leads to a stable free radical (as is the case with vitamin E, and other commercial antioxidants).

In addition, the biological properties of DHII and its analogues were to be examined by Shertzer's group in the USA. Collectively, these activities were aimed at the identification of a novel candidate antioxidant drug.

3:2 Preparation of dihydroindeno[1,2-*b*]indoles

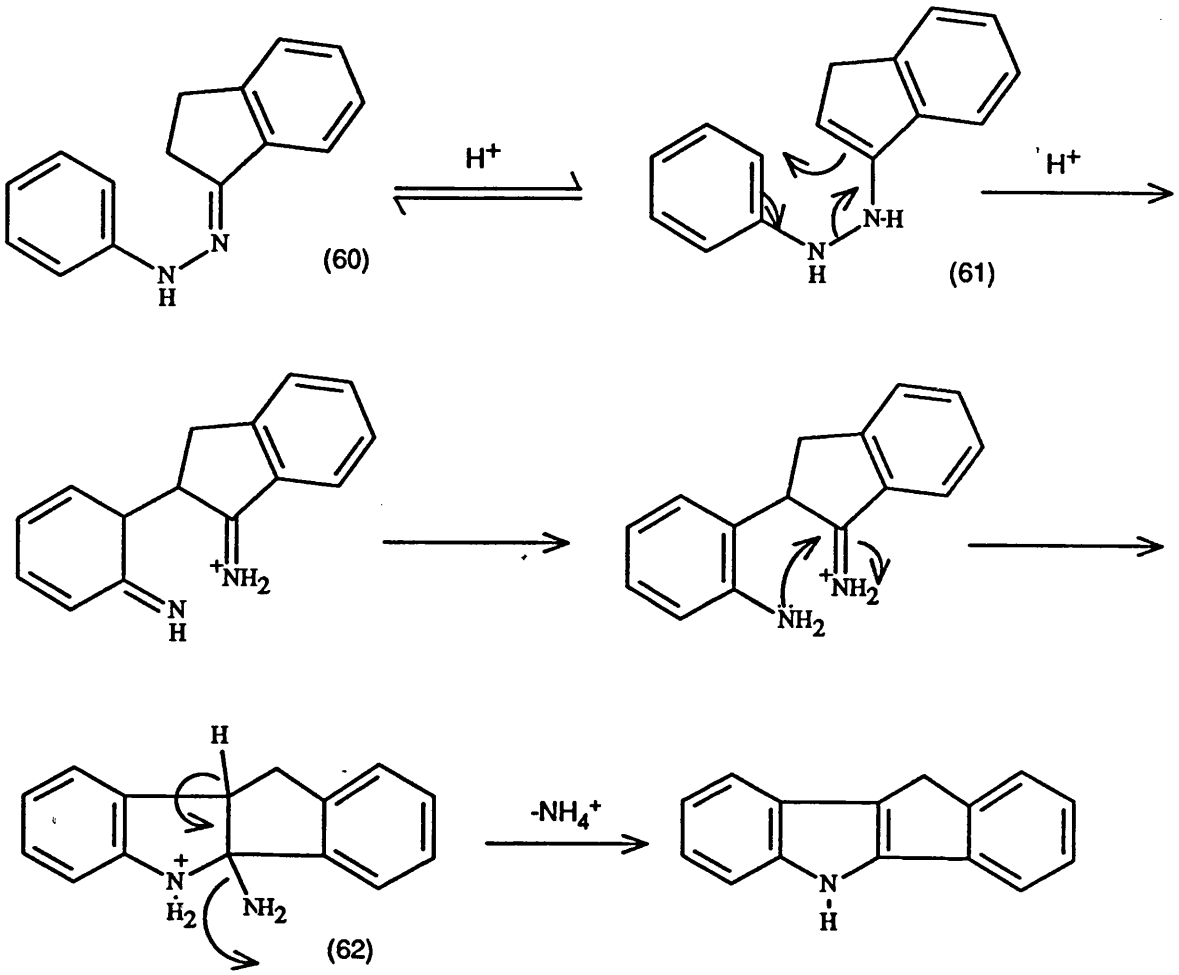
The synthesis of DHII was first described in the chemical literature by Hausmann in 1889[2] *via* a Fischer–indolisation procedure using concentrated hydrochloric acid as

catalyst. His work was repeated by Kipping,[3] and also by Leuchs *et al.*[4]

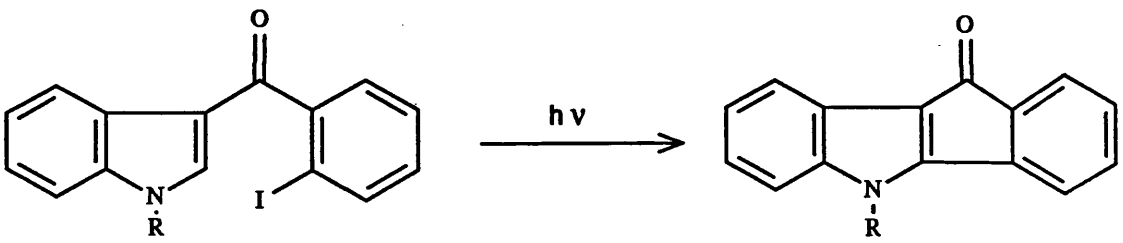
The Fischer reaction is well known for the synthesis of indoles, and much work has been undertaken [5-7] in order to deduce the mechanism of the reaction, which is now considered to involve a [3,3] sigmatropic shift within the ene–hydrazine tautomer (61) of the phenylhydrazone (60) (scheme 3:1). Rearomatisation of the product of the rearrangement, is followed by intramolecular ring closure to give the aminoindoline (62). This species then eliminates ammonia to furnish the indole. The main role of the acidic catalyst (which need not be hydrochloric acid) in this reaction appears to be in improving the rate of conversion of (60) to (61), although protonation is not always required, and the reaction may proceed under thermal conditions only (the example shown in scheme 3:1 is that of the synthesis of DHII).

An alternative approach to the indeno[1,2-*b*]indole system has been reported by Carruthers.[8] Here a photochemical cyclisation of 3-(2-iodobenzoyl)indole (63) affords indeno[1,2-*b*]indol-10(5*H*)-one (64, R=H) (scheme 3:2). In a similar manner, Itahara has cyclised 3-benzoyl-1-methylindole to yield 5-methylindeno[1,2-*b*]indol-10(5*H*)-one (64, R=Me), using palladium acetate as a cyclisation catalyst, in 66% yield.[9] However, the evidence provided to prove that the linear ketone (64, R=Me) had formed, was sparse, and no comparison with the authentic compound was made. There is, of course, the possibility of cyclisation to the angular isomer (65), and so Itahara's result was queried by Sainsbury *et al.*,[10] especially since Itahara himself had indicated that a change in the substitution pattern of the substrate, alters the course of the reaction. In the event, Itahara was proved correct in the assignment of the product as the linear ketone (64, R=Me). Unfortunately, there are difficulties in the reduction of this ketone,[11] and the cyclisation is also unreliable. These factors do not make this route an attractive alternative for the large scale preparation of DHII.

For this, the Fischer synthesis is very suitable, and requires the reaction of 1-indanone, and phenylhydrazine hydrochloride, in glacial acetic acid as solvent. The pro-



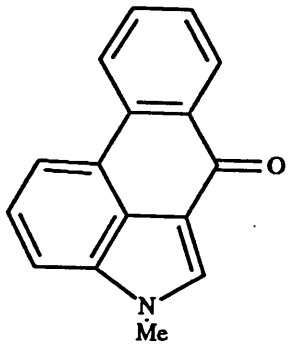
Scheme 3:1



(63) (64)

Scheme 3:2

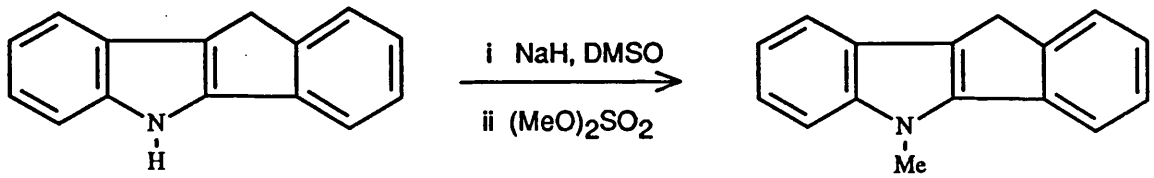
duct precipitates out of the hot acetic acid, and impurities are then removed from the crude compound by boiling it in absolute ethanol. DHII remains insoluble in this solvent and is readily collected. In this manner, yields of up to 90% of the pure compound may



(65)

be achieved.

In chapter 1 it was suggested that the nitrogen atom plays an important role in the mechanism of antioxidation in the indole series, implying that the radical produced in oxidation is centred on the heterocyclic ring. Therefore the effect of *N*–methylation was investigated. *N*–Methyl–DHII (66) was prepared from DHII by base promoted alkylation (scheme 3:3). In this type of reaction, indoles may alkylate at either the 1 or the 3 position, but when sodium or potassium ions are used as counter cations, it is normally found that *N*–alkylation predominates. This was the case here, and when DHII was treated with dimethylsulphate and the sodium salt of DMSO used as base, the *N*–methyl compound was formed in 53% yield.



(66)

Scheme 3:3

In the autoxidation assay, *N*–methyl–DHII proved to be rather less efficient than DHII itself (see table 3:1). This could mean several things, for example:

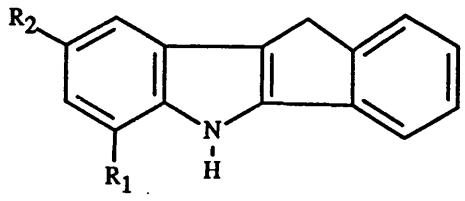
i) an increase of steric resistance to the approach of the oxidant;

ii) an adverse change in ionisation potential;

iii) an increase in the lipophilicity of the substrate.

Clearly, *N*–methylation should change the ionisation potential of DHII, and so we next

investigated the effect of substitution in ring A, either with electron donating, or with electron withdrawing groups. Three substituted compounds were prepared; these being: 8–fluoro– (67), 8–methoxy– (68), and 6–chloro–DHII (69).



(67) $R_1 = H$, $R_2 = F$

(68) $R_1 = H$, $R_2 = OMe$

(69) $R_1 = Cl$, $R_2 = H$

8–Fluoro–DHII (67) was synthesised from 1–indanone and 4–fluorophenylhydrazine by another worker at Bath University. 8–Methoxy–DHII (68) was similarly prepared from 1–indanone and 4–methoxyphenylhydrazine hydrochloride, using PPE[12] as catalyst for the ring cyclisation,[13] in a yield of 78%. PPE was also used to prepare 6–chloro–DHII (69) in 82% yield. It was found that this derivative could also be prepared by pre–adsorbing the *o*–chlorophenylhydrazone of 1–indanone onto silica, and heating it to 140°C. This technique could not be used to such good effect to prepare other DHII compounds such as the parent (20), or 8–methoxy–DHII (68), but in the case of (69), this particular preparation lends itself well to direct purification of the product formed, by simply adding the silica from the reaction onto the top of a column made up from fresh silica, and eluting with 5% ethyl acetate/petrol (60–80°C).

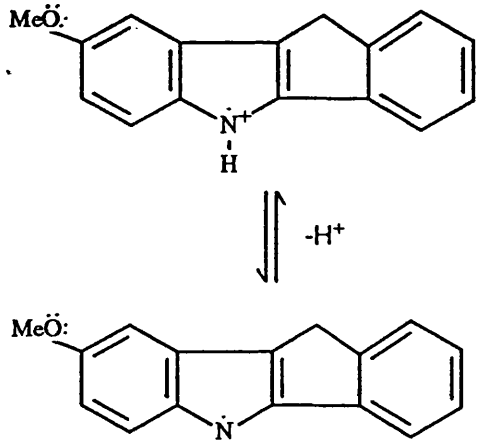
Heterogeneous catalysts have been used in Fischer–indole syntheses on many occasions, the most recent results being discussed in the Russian literature.[14] Silica may be acting here as an acid catalyst, as well as a template – maintaining the ene–hydrazine in the ideal conformation to aid cyclisation. In this situation, a small change in the substitution pattern could perhaps alter the way in which the intermediate is held by the silica, thus producing or impairing cyclisation.

The results of the phospholipid–oxidation inhibition assay for the substituted DHII compounds are given in table 3:1. Activity was decreased in all of the compounds except for 8–methoxy–DHII (68).

Table 3:1

Biological results on substituted DHII	
Compound	50%I (Fe) μM
DHII (20)	1.5
5-methyl-DHII (66)	8.5
8-fluoro-DHII (67)	2.5
8-methoxy-DHII (68)	0.65
6-chloro-DHII (69)	7.0

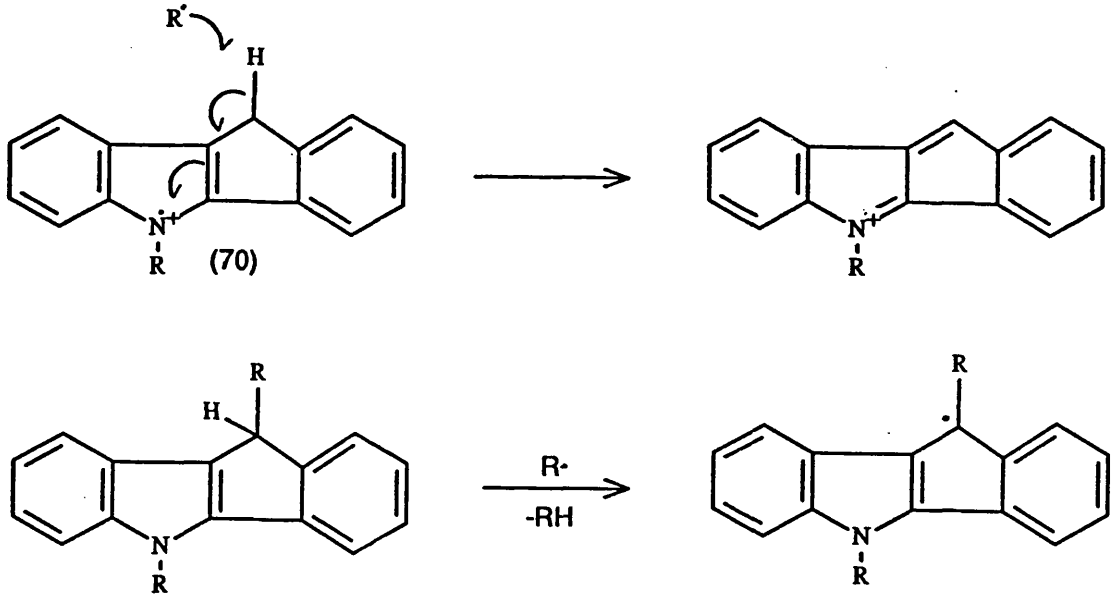
It could be argued that the increase in activity for the methoxylated compound is associated with the stabilisation of the cation radical (or radical) formed by the loss of an electron (and a proton) from the nitrogen atom of the indenoindole system (scheme 3:4). However, the mesomeric stabilisation of the fluoro substituent in the 8-fluoro- derivative, should be offset by the inductive pull of the halogen atom. What is surprising is that this compound is still quite active - more so than 5-methyl-DHII.



Scheme 3:4

A second problem is that the radical cation (or radical) formed, although delocalised, does not appear particularly stable relative, say, to the phenoxy radical of BHT (see

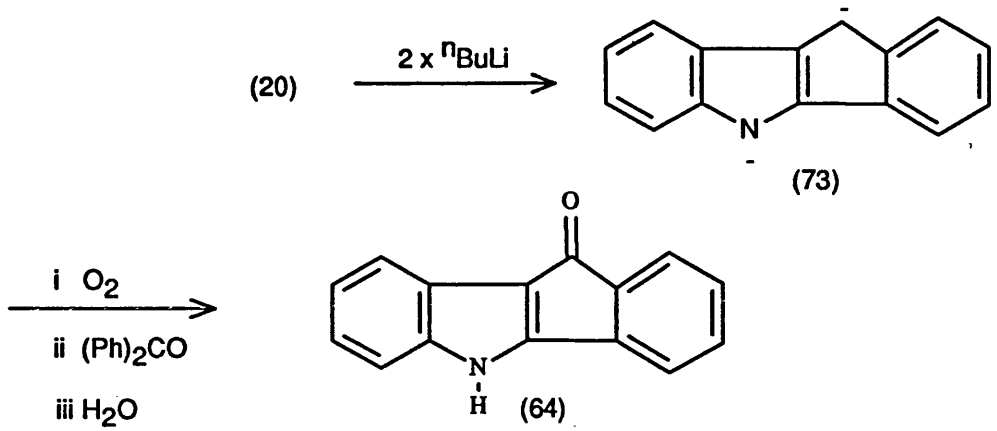
chapter 1), it is not, for example, sterically crowded and thus should react further with other radicals, or with nucleophiles. There is obviously more to be learnt, and next the effect of varying the substituents on the methylene bridge was investigated. For example, the radical. or the radical cation (70) may undergo further reactions involving the loss of a hydrogen atom, or a proton from this unit (scheme 3:5). If so what would be the effect of blocking or partially blocking this process? An alternative mode of action for DHII and its analogues might be that they simply react with radicals by hydrogen atom transfer. In this case *N*–methylation would preclude reaction at the nitrogen atom, but substitution at either *ortho* or *para* positions in the ring should help stabilise the radical formed in the order MeO > Cl > F (reflecting the availability of the lone pair electrons). That the *N*–methyl compound is active would then suggest that there is an alternative site for hydrogen atom abstraction *ie* at C–10 (scheme 3:5).



Scheme 3:5

To assess this last possibility, four compounds are needed: 10–methyl–DHII (71), 10,10–dimethyl–DHII (72), and their *N*–methyl derivatives (see back of p35).

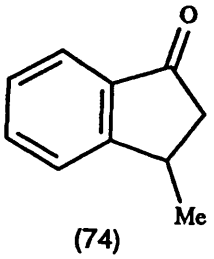
Eisch and Abraham have described[15] an experiment in which the dianion of DHII (73) was obtained through the action of ^{n}BuLi on DHII. This anion was then treated with oxygen and allowed to transfer hydride to benzophenone, to form the ketone (64)



Scheme 3:6

(scheme 3:6).

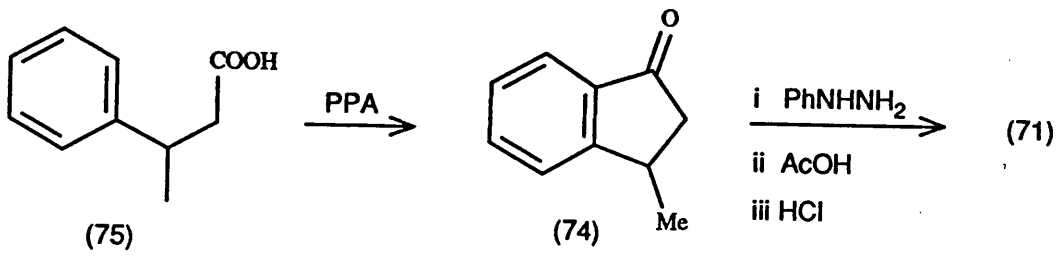
Even though we did not require di-alkylation initially, a supply of the ketone was desirable since it would be possible to convert it to (71) or (72) by conventional methods. However, in our hands, Eisch and Abrahams' procedure gave the blood-red ketone in only 8% yield, along with other unidentified products. Therefore attention was given to the preparation of (71) *via* the Fischer-indole synthesis using 3-methyl-1-indanone (74).



(74)

This ketone was prepared by the cyclisation of 3-phenylbutyric acid (75) by polyphosphoric acid (PPA). The indanone was heated to reflux in glacial acetic acid with phenylhydrazine, and then concentrated hydrochloric acid was added to catalyse the cyclisation. This afforded the indole in 43% yield after purification (scheme 3:7).

Intuitively, monomethylation at C-10 would not be expected to cause a reduction in activity, since loss of a hydrogen radical is still possible at this site, and the radical formed is tertiary rather than secondary. 10-Methyl-DHII is, however, less active than its parent (table 3:2), and this caused us to pause and investigate a reason for this



Scheme 3:7

apparent anomaly.

Table 3:2

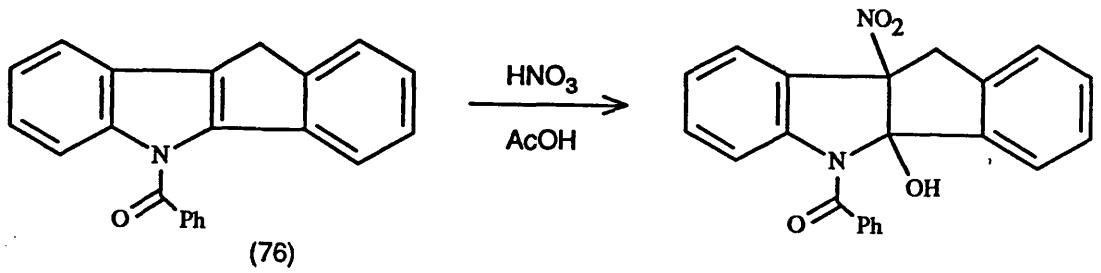
Biological results on 10–Me–DHII	
compound	50% I (Fe) μM
DHII (20)	1.5
10–methyl–DHII (71)	2.0

3:3 Chemistry of DHII

3:3:1 *Previous work*

There is a paucity of information in the literature concerning the physical and chemical properties of DHII, however, 5–methyl–DHII (66) has found some use as an organic scintillator,[16–18] for use in the detection of fast electrons.[19]

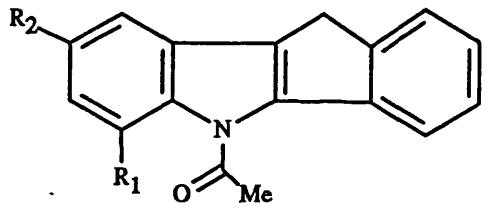
The earliest reference concerning a chemical transformation of a derivative of DHII, involves the action of nitric acid on 5–benzoyl–DHII (76).[20] Basing their results on observations on similar reactions on tetrahydrocarbazole, Bryant and Plant claim that the nitric acid adds across the indole double bond (scheme 3:8). This structure has not been checked since the derivative was made in 1931.



(76)

Scheme 3:8

In a later paper that same year, Plant investigated the action of bromine in acetic acid on 5–acetyl–DHII (77),[21] obtaining a mixture of 6–bromo– (78), and 8–bromo–DHII (79).

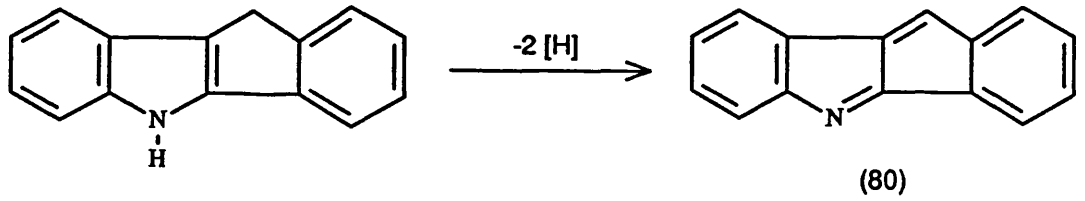


(77) $R_1 = R_2 = H$

(78) $R_1 = H$, $R_2 = Br$

(79) $R_1 = Br$, $R_2 = H$

There are a number of papers in the literature concerning the attempted dehydrogenation of DHII to yield indeno[1,2–*b*]indole (80), also called dibenz[*b*,*f*]azapentalene (scheme 3:9). This molecule contains 16π electrons, and is formally antiaromatic, consequently, it is unlikely to be a stable compound.

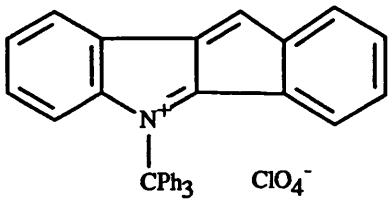


(80)

Scheme 3:9

Reid attempted the dehydrogenation of (20) with triphenylmethyl perchlorate[22] and claims that the product (80) quaternises with excess reagent to give 5–(triphenylmethyl)indeno[1,2–*b*]indolium perchlorate (81) as a black solid. Since the species (80) is already electron deficient, a further acquisition of positive charge is not

particularly helpful, and such a structure seems improbable.

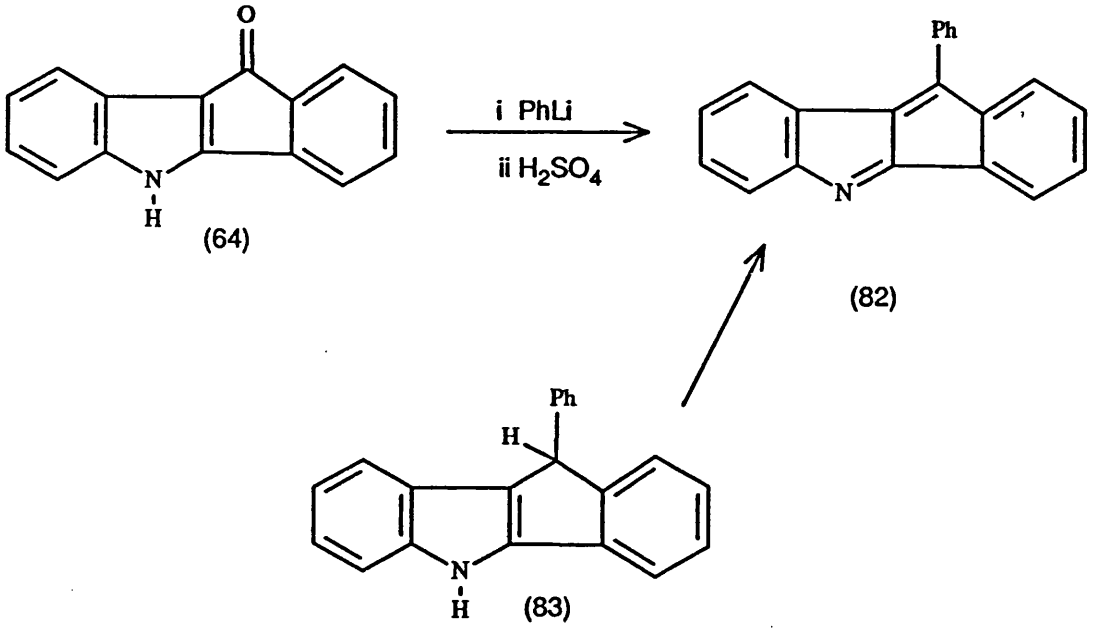


(81)

Kempter *et al.* attempted to prepare the azapentalene (80) by dehydrogenation of (20) with quinoline and obtained a highly coloured solid.[23] Little evidence was provided by the authors to substantiate the claim that the antiaromatic compound had been formed. We suggest that the colours alone suggest that the proposed structures are incorrect, since Eisch and Abraham have shown[15] that 10–phenylindeno[1,2–*b*]indole (82) is golden brown in colour – this compound is of course more highly conjugated than (80), therefore the simple azapentalene would be expected to absorb at lower wavelengths.

10–Phenylindeno[1,2–*b*]indole (82) has been properly characterised, and is synthesised, either by reaction of the ketone (64) with phenyllithium, followed by dehydration of the product alcohol, or from the dehydrogenation of 10–phenyl–DHII (83) with 2,3–dichloro–5,6–dicyano–1,4–benzoquinone (DDQ), (scheme 3:10). It must owe its existence to the aforementioned delocalisation with the phenyl group, which diminishes its energy content. Also the phenyl substituent would be expected to sterically hinder reaction at the 10 position, one of the sites through which the azapentalene is expected to "dimerise".

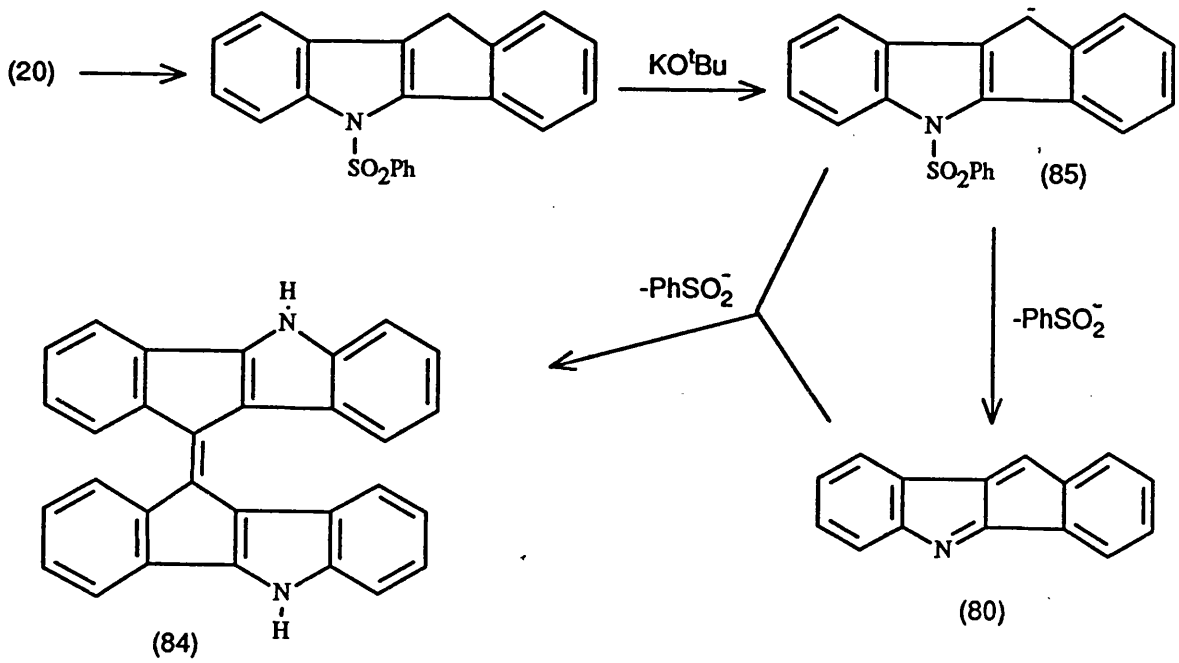
We have reacted DHII with triphenylmethylfluoroborate, to obtain a black amorphous solid, which is insoluble in most organic solvents. This is similar to the product described by Reid, but it seems not to be a single product as the ^1H nmr spectrum in CF_3CO_2D as solvent is very complex, and exhibits numerous unresolved signals in the aromatic region. Similarly, this black solid is partially decolourised by treatment with



Scheme 3:10

sodium borohydride in ethanol to produce a mixture containing many spots by t.l.c. but none of these is the DHII starting material. Examination of the spectrum of the mixture, suggest that it contains some dimeric material perhaps similar to structure (84), a product described by Eisch and Abraham in 1976[15] from the reaction of *N*-benzenesulphonyl-DHII with potassium *tert*-butoxide. Eisch and Abraham indicate that this compound is formed by the base promoted loss of benzenesulphinic acid leading to dibenz[*b*,*f*]azapentalene (80) which then reacts with the anion (85) formed by deprotonation of the same starting material at C-10. Thereafter, further elimination of benzenesulphinic acid affords the dianion, which on work-up leads to the suggested product (scheme 3:11).

Abraham has attempted to prepare the parent indeno[1,2-*b*]indole (80) by electron transfer from the dianion of DHII (73) using *N*,*N*-diethyl-*O*-mesitylenesulphonylhydroxylamine (86),[24] or other similar reagents.[25] Here, electron transfer from the dianion to the sulphur atom of the reagent, leads to the reagent fragmenting, releasing a diethylamine radical and the formation of the radical anion (87).

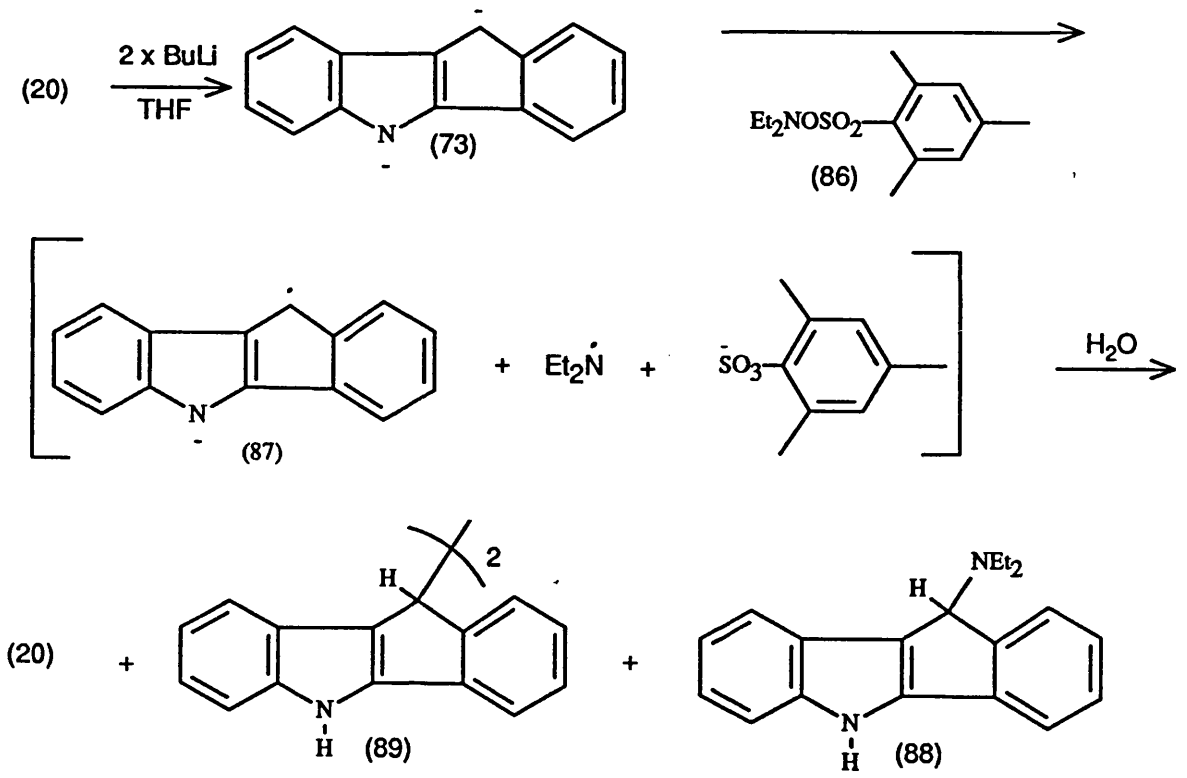


Scheme 3:11

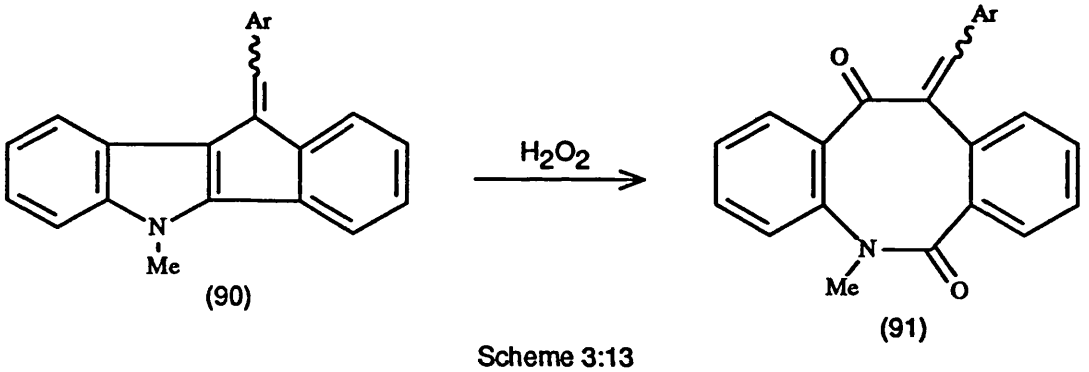
These two products couple to yield 10–diethylamino–5,10–DHII (88). Also obtained was the 10,10'–dimer (89) produced by coupling of the radical anion (87) – (scheme 3:12). This dimer was identified by ^1H nmr, and by mass spectrometry[25] but was not isolated and properly characterised. It seems to exist in two forms – the *meso* form, and a racemate. These two forms were separable by HPLC, although Abraham does not attempt the separation on a preparative scale.

In 1987, Letcher published details of a reaction between benzylidene derivatives of DHII (90), and hydrogen peroxide[26] which cleaved the 4b–9b carbon–carbon bond to give 5,11–dioxodibenz[*b*,*f*]azocines (91) in 100% yield (scheme 3:13).

Interestingly, this reaction fails in our hands when applied to DHII itself suggesting that the exocyclic double bond in Letcher's substrates reduces the aromaticity of the indole nucleous. It is known, however, that under more vigorous treatment, other indoles lead initially to 3–hydroperoxides which may then breakdown to oxyradicals which rearrange to indoxyls or oxindoles on reaction with hydrogen peroxide.[27, 28]



Scheme 3:12



Scheme 3:13

3:3:2 *Attempted oxidation of DHII*

It was suggested in chapter 1 that *in vivo* the dietary indoles modify the response of the microsomal monooxygenase system, particularly that of cytochrome P450. At the beginning of this project, a paper by Tabushi and Morimitsu described a reaction medium designed as an *in vitro* model of P450.[29] This medium is effective in monooxygenating phenols and similar aromatic systems. We sought to investigate the effect of this system on one of the simple dietary indoles 3,3'–diindolylmethane (3) in the hope

that we might mimic its metabolic fate.

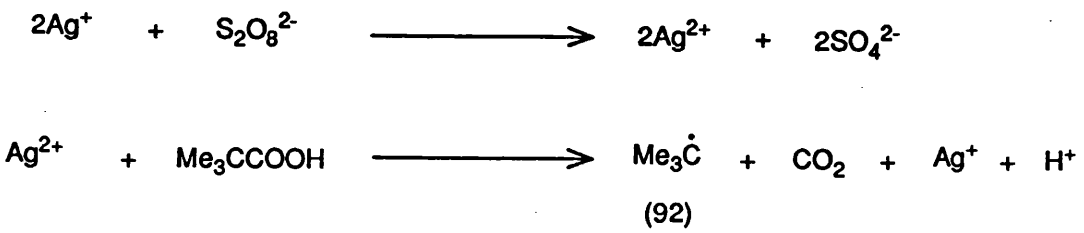
The model oxidase system consists of a metalloporphyrin – tetraphenylporphyrin containing manganese(II)chloride – and is generated by the reaction of pyrrole and benzaldehyde in hot propionic acid,[30] followed by treatment with manganese(II)chloride in DMF at reflux.[31] The remaining elements of the "oxidase", are *N*–methylimidazole, a platinum catalyst (supported on polyvinylpyrrolidone), all maintained under an atmosphere of hydrogen and oxygen, with ethanol as solvent.

Unfortunately, this mixture,when applied to the di–indolylmethane (3), caused overoxidation, and the formation of a number of unidentifiable products.

3:3:3 *Action of radicals on DHII*

The effect of treating DHII with sources of radicals was next investigated by us, in the hope of detecting any stable radicals which might form from it.

In a preliminary experiment, DHII was treated with *tert*–butyl radicals (92), generated by the action of silver persulphate upon 2,2–dimethylpropanoic acid (scheme 3:14).[32]

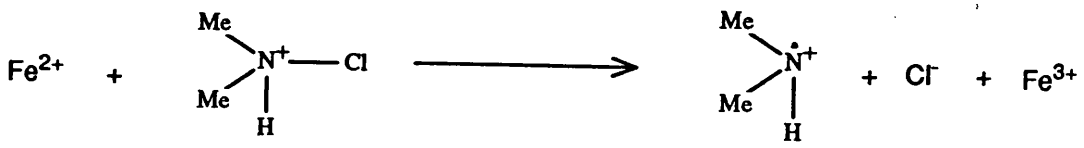


Scheme 3:14

Disappointingly, the substrate was unaffected by these conditions, and 95% of the DHII was recovered unchanged from the reaction.

A more promising reaction was observed when DHII was reacted using conditions described by Minisci,[33] which involve the iron(II) catalysed dechlorination of chlorodi–

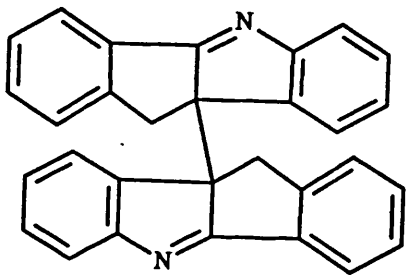
methylamine (93) in sulphuric/acetic acid (3:1), to give the dimethylamine radical cation (43), (scheme 3:15).



(93) (94)

Scheme 3:15

A major product was identified by t.l.c., which, after purification by column chromatography, was isolated as a white solid, m.p. >280°C (dec. at 220°C). This compound was characterised as the 9b,9b'–dehydrodimer (95), yield 20%.



(95)

It has the following physical data:

i) m/z (M^+): 408,

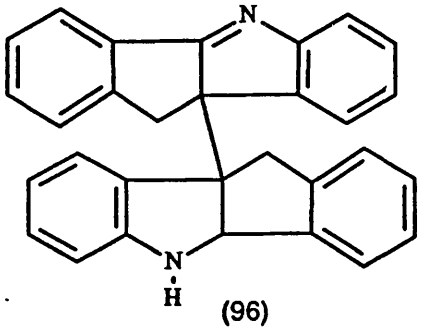
ii) ^{1}H nmr δ_H ($CDCl_3$): 2.72 (2H, d, ^2J 17.6Hz), 3.46 (2H, d, ^2J 17.6Hz), 6.8–8.1 (16H, m). The ^{1}H nmr spectrum indicates that the dimer has a centre of symmetry – each half being equivalent. In line with the structure (95), the two protons on each methylene bridge are non-equivalent, one pointing "into" the dimeric structure, and the other away. As a result, each pair generates an AX spin–spin system with a coupling constant J = 17.6Hz.

iii) λ_{max}^{EtOH}nm(ϵ): 203 (63830), 240 (33190), 248 (34722), 287 (24510), 315 (27063). The uv spectrum of the dehydrodimer alters on the addition of sodium borohydride; the absorbance at λ_{max} 205nm increasing w.r.t. the other absorbances, this indicates that the imine double bonds are saturated by this treatment, and the

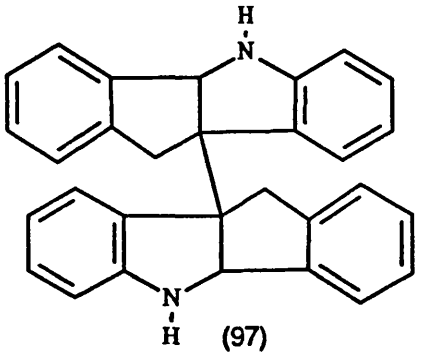
chromophore reverts to that of a simple benzene type.

iv) Microanalysis: found C, 88.3; H, 5.13; N, 6.72; $C_{30}H_{20}N_2$ requires: C, 88.2; H, 4.93; N, 6.85%.

v) Treatment with 1–2 equivalents of sodium cyanoborohydride in a solution of citric acid in ethanol (pH 6) gave mixture of two products; m/z (M^+): 410 and 412. These are the semi-reduced, and the fully reduced compounds (96) and (97) respectively.



(96)



(97)

To aid assessment of the reaction mechanism, the following test reactions were undertaken, and the following results obtained:

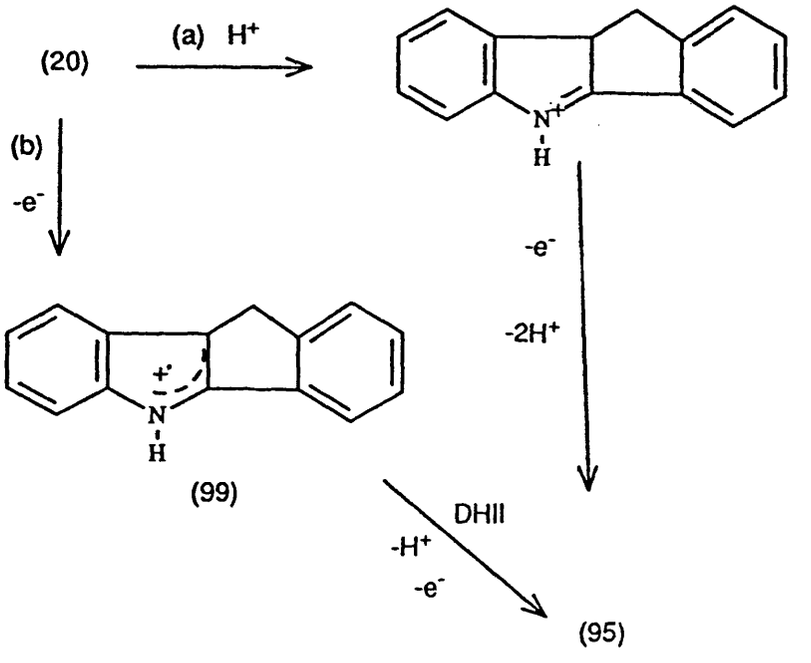
i) on addition of sulphuric acid/acetic acid alone, DHII can be recovered in 100% yield after a number of days,

ii) addition of Fe(III) ions alone at varying pH, no dehydrodimer was formed from DHII, therefore one electron oxidation by Fe(III) does not lead to the coupling reaction.

iii) reaction with another strong source of radicals – *tert*-butyl peroxide – gave traces of the dehydrodimer, but only in the presence of a strong acid.

Therefore a possible mechanism (a) for the formation of (95) involves initial protonation of DHII at the 9b position, followed by abstraction of an electron to give a radical cation (98), this species then dimerises and loses two protons to give the dehydrodimer (95), (scheme 3:16). The radical cation (98) is probably stabilised by the presence of a

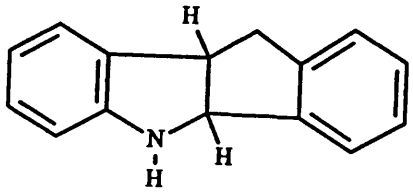
non-polarisable anion (HSO_4^-). Such results are known for radical cations generated through electrochemical oxidation in the presence of trifluoroacetates as supporting electrolytes. Parker has suggested that the presence of a non polarisable counter ion is not essential for the formation of radical cations, but simply extends their life times, and thereby promotes certain intermolecular changes.[34] Without this argument it is hard to judge why sulphuric acid is necessary for, of course protonation is not essential for the preparation of a radical cation (99), which could be directly formed by one electron oxidation of DHII (scheme 3:16, route b).



Scheme 3:16

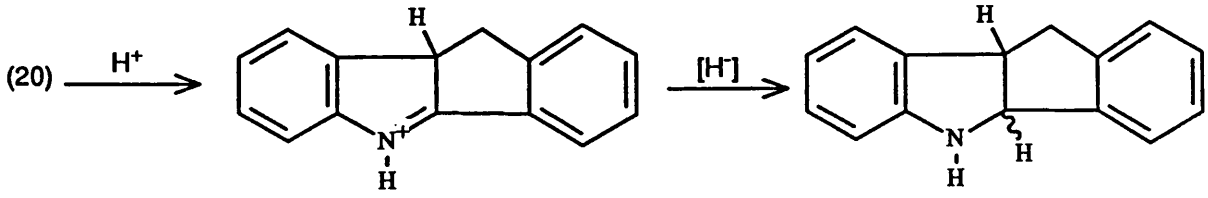
Obviously, this point needs clarification, and we sought a means of examining the result of radical cation production by electrochemistry. The results of this work are discussed in section 4:2:1, together with similar experiments carried out on other compounds in the series.

One interesting, but perhaps coincidental point was noticed during the electrochemical experiments (section 4:2:1), this is the relationship between the first ionisation potential of the substrate (electron loss from whatever π source), and the activity of the substrate in the Fe(III)/ascorbic acid test. If one considers ionisation from rings A/B (the indole moiety), it is obvious that electron loss from the dihydro (indoline) derivatives will be easier. It was this reasoning which lead us to synthesise some 4b,5,9b,10-tetrahydroindeno[1,2-*b*]indoles (*e.g.* THII, 100). The discovery, and subsequent interest which these compounds has generated, has overridden virtually all of our early plans, including some of the structure/activity analyses which we had hoped to do with the simpler compounds.



(100)

There are numerous methods for reducing indoles to indolines, but we found that DHII could be reduced in large scale, and in very high yield, by treatment with sodium cyanoborohydride in glacial acetic acid at room temperature: conditions described by Kumar and Florvall[35] in 1983. This reaction is thought to proceed by addition of the hydride onto the intermediate iminium ion formed by protonation on the acid medium, and so theoretically could produce both *cis* and *trans* isomers of THII (scheme 3:17).



(100)

Scheme 3:17

Examination of models of THII suggests that from a steric viewpoint, the *cis* configuration must predominate, even though from an electronic argument, the *trans*

form is equally possible. Models of the two configurations of THII (see appendix 1), show that the dihedral angle H_{4b}–C–C–H_{9b} is about 1°C (*cis*), or 179°C (*trans*). Use of the Karplus equation suggests that for the *cis* compound this would give a value for the $^3J_{H_{4b},H_{9b}}$ coupling constant of 8.0–8.5Hz, whereas in the case of the *trans* compound, the coupling constant would be 9–10Hz. Examination of the ^{1}H nmr spectrum of THII yielded a value of $^3J_{H_{4b},H_{9b}}$ of 8.4Hz, which suggested that the product of the sodium cyanoborohydride reduction of DHII did have *cis* geometry. Inspection of the other dihedral angles between C_{9b} and C_{10} also yielded similar findings.

However, the Karplus equation is only truly reliable in the case of simple cyclohexane derivatives, and so additional evidence was required. This came in the form of a single crystal X-ray experiment (see appendix 1), which showed as expected that the product did indeed have *cis* geometry across the 4b–9b bond.

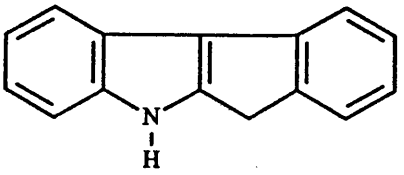
THII was assayed for antioxidative activity using the iron/ascorbic acid system (table 3:3), and found to exhibit 10 times more activity than DHII (20).

Table 3:3

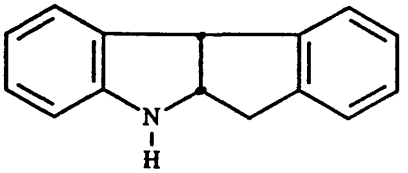
Activity of THII	
compound	50% I (μM)
DHII (20)	1.4
THII (100)	0.15

This observation made the THII family of compounds very worthy of further study. In addition, we deemed it necessary to examine the isomers (101) and (102) of DHII and THII respectively. An examination of these last compounds might exemplify the role of the methylene group, and the nature of the ring fusion in our substrates.

In addition, we wished to examine the effect of *N*–substitution in the THII series,

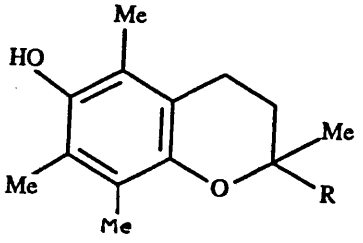


(101)



(102)

and that of substitution in ring A. From the standpoint of pharmacological optimisation, the presence of a lipophilic group in THII, such as the phytyl chain in α–tocopherol, might be useful. This branched 16–carbon alkyl chain might locate the antioxidant chromophore on the surface of cell membranes. [36] There is some evidence for this since, if α–tocopherol (103) is incorporated within a fully saturated liposome (dimyristoyl phosphatidylcholine), and added to an aqueous dispersion of soybean liposome (an unsaturated oxidisable system), the system is unable to inhibit the oxidation of the soybean preparation, whereas a related molecule 2,2,5,7,8–pentamethyl–6–chromanol (PMC, 104) which does not have this hydrocarbon tail, was able to act as an inhibitor.



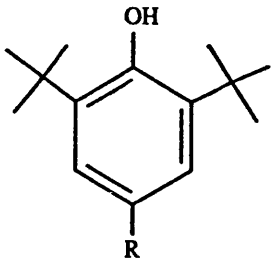
(103) R = $C_{16}H_{33}$

(104) R = Me

This result is explained if the lipophilic tail of (103) holds the antioxidant in the membrane of the first cellular system, not allowing it to pass from cell to cell. On the other hand PMC moves freely within the system. Thus although the lipophilic tail has little effect on the ionisation potential of vitamin E, the effect of binding the molecule within a cell membrane may well enhance its efficiency as a biological antioxidant by placing it at points most susceptible to damage by invasive radicals.

Similar results were obtained when the same authors compared, under identical conditions to the vitamin E experiments, the efficiency of 2,6–di–*tert*–butylhydroxytoluene (BHT, 6), and the more lipophilic stearyl–(2,6–di–*tert*–butyl–4–

hydroxyphenyl)propionate (105) as cellular antioxidants.



(6) R = Me

(105) R = $(CH_2)_2COOC_{18}H_{37}$

In view of these discoveries, and in terms of ease of synthesis, derivatives bearing a lipophilic group attached to the *N*–atom of THII were targeted, and we added the production of these to our list of objectives.

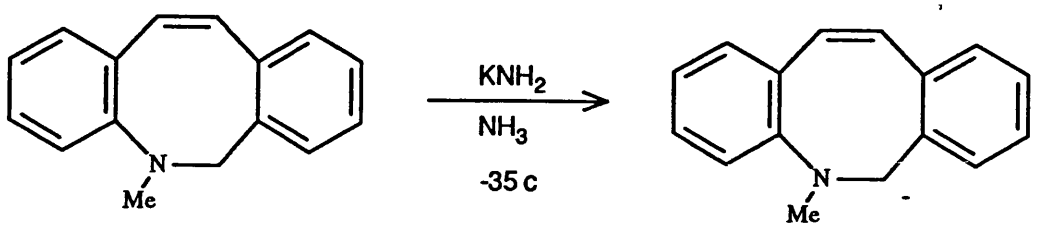
Two other aims were to study the effect of substituting certain aryl (ring A) protons, and those of rings B and C by simple alkyl groups. Not only would this raise the lipophilicity of the molecule as a whole, it would also help stabilise any radical cation (or radical) formed by hyperconjugation (at certain sites), and by steric shielding. In addition, substitution in rings B/C would prevent hydrogen atom abstraction which could actually inhibit activity.

Additionally, other compounds were needed bearing a range of substituents, either as controls, or in an attempt to "tune up" the structure in order to find the most active compound. Also, we wanted to try and find some chemical means to produce a radical cation or a radical from THII so as to study its fate outside of a biological system, these latter attempts are described in chapter 4.

3:4:1 *Literature references of THII*

A study of the literature for references to compounds containing the 4b,5,9b,10–THII ring system, finds only a single paper. In this, Anastassiou and co–workers[37] – as part of a project to study potentially aromatic 18π electron systems – took 5,6–

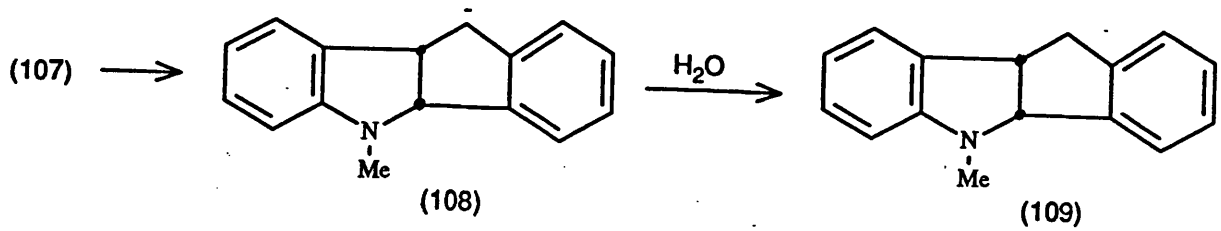
dihydro-5-methyldibenz[*b*,*f*]azocine (106) and formed from it, the conjugate base (107) by treatment with potassium amide in liquid ammonia at -35°C (scheme 3:18).



(106) (107)

Scheme 3:18

However, the ^{1}H nmr spectrum of this anion recorded at a temperature of 0°C indicated that it had rearranged into the tetracyclic tautomer (108), which upon protonation affords 4b,5,9b,10-tetrahydro-5-methylindeno[1,2-*b*]indole (109), (scheme 3:19).



(108) (109)

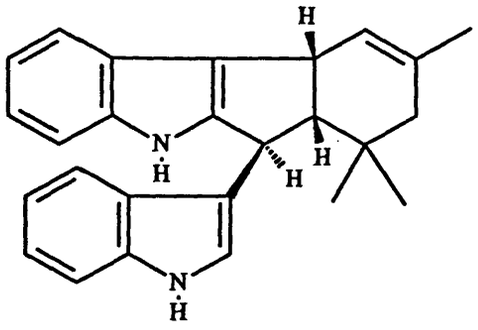
Scheme 3:19

Although the anion (107) is formally a ring system containing $4n+2\pi$ electrons, apparently, it may not attain the necessary planarity for stability, and it prefers to adopt the angular tetracyclic structure (108).

The *cis* geometry of the product (109) was determined by studying the coupling constants from the ^{1}H nmr spectrum, and comparing these with dihedral angles measured on a molecular model.

3:4:2 *Preparation of* iso-*THII*

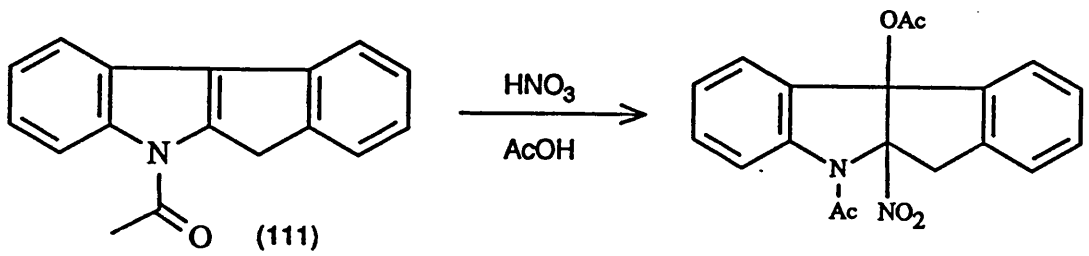
There is even less information concerning compounds bearing the indeno[2,1-*b*]indole chromophore (for example 5,6-dihydroindeno[2,1-*b*]indole, *iso*-DHII, 101), than



(110)

there is for DHII and THII. Apart from the plethora of publications concerning the natural product yuehchukene (110),[38–40] (see back of p52) the series is not cited Thus *iso*–THII and its derivatives are novel compounds.

Iso–DHII (101) was first reported in 1922 independently by Cawley and Plant,[41] and by Armit and Robinson.[42] Both sets of authors obtained ths compound from the Fischer cyclisation of the hydrazone of 2–indanone. Plant, in a later publication,[43] relates the action of nitric acid in acetic acid on *N*–acetyl–*iso*–DHII (111) stating that it produces products similar to those proposed for the nitration of DHII under the same conditions (see section 3:3:1) (scheme 3:20).



Scheme 3:20

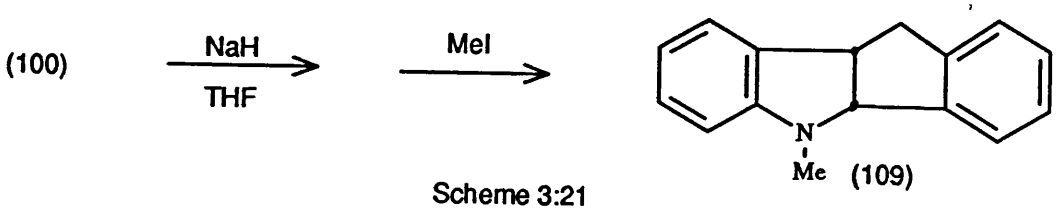
Apart from a brief mention by Sainsbury *et al.* in their paper concerning the cyclisation of 3–benzoylindoles[10] (see section 3:2), there are no other references to this class of compounds.

In our hands, *iso*–DHII (101) was prepared in 8% yield from 2–indanone and phenylhydrazine hydrochloride in glacial acetic acid, and this product was then reduced using sodium cyanoborohydride in acetic acid to give *iso*–THII in 43% yield. The yields were not optimised, as these compounds upon biological assay showed no improvement upon DHII or THII as antioxidants (see section 4:1).

3:4:3 N-*substitution of THII*

N–Methyl–THII (109) was prepared by the base promoted alkylation of THII. Sodium hydride or *n*–butyllithium were used as bases with THF as solvent, and

alkylation was achieved by the addition of iodomethane to the solution of the anion. This afforded the desired product in quantitative yield (scheme 3:21).



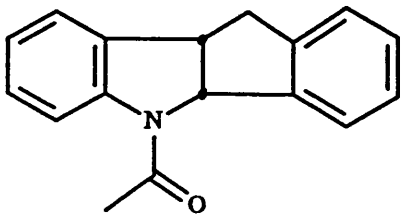
Scheme 3:21

It had identical physical properties to the compound prepared by Anasstassiou,[37] and described in section 3:4:1.

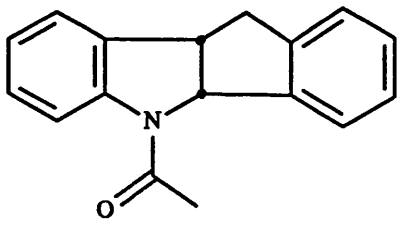
In section 3:2, it was shown that the effect of *N*-methylating DHII, was to lower its biological activity. However this was not the case with *N*-methyl-THII which was assayed as the best compound in the indenoindole series at that time (table 3:4).

Interestingly, *N*-acetylation of THII has the opposite effect, and in the Fe(II)/ascorbic acid test, activity is lost. This suggests that there is a critical oxidation potential for antioxidant effect, for it is expected and observed that *N*-acylation depletes the electron density within the π system of ring A of the THII chromophore.

N-Acetyl-THII (112) was prepared in a similar fashion to *N*-methyl-THII, using acetyl chloride in place of iodomethane. The ^{1}H nmr spectrum of this compound showed it to consist of a mixture of the *E*/*Z* isomers (112a and 112b).



(112a) E



(112b) Z

This result can be demonstrated by using the technique of saturation transfer. For example, the two resonances for the proton α to the nitrogen (4b-H) are separated by *ca.* 0.5ppm. Presaturation of one of these resonances in an transient nOe difference experi-

ment saturates the other resonance (figure 3:1), indicating that the two resonances are in fact due to the same proton in two geometric isomers that are exchanging slowly on the nmr timescale.

In figure 3:1, the resonance of H-4b for one of the isomers at 5.8ppm was irradiated (arrowed in the upper - control - spectrum), and complete transfer of saturation to the other geometric isomer, was shown by the fact that in the difference spectrum, the resonance due to H-4b at 6.3ppm, is inverted to the same extent as at the irradiated position. This difference plot also shows an nOe (*ca.* 15%) from H-4b to the resonances due to H-9b at 4.2 and 4.3ppm. This provides additional proof that the THII family of molecules has a *cis* ring fusion between rings B and C.

The activities of the *N*-substituted THII compounds are listed in table 3:4. As it can be seen, the *N*-methyl derivative was the most active compound at the time, while the *N*-acetyl compound was virtually inactive.

Table 3:4

Activity of *N*-substituted THII	
compound	50% I (μM)
THII (100)	0.140
N-Me-THII (109)	0.062
N-Ac-THII (112)	1800+

Sainsbury argued that for increased pharmacological effect a lipophilic "tail" was required. This would have the effect of binding the compound to the "fatty" cell membrane, leaving its relatively polar head available to capture any invasive and damaging oxygen radicals. As a preliminary attempt to investigate this idea, we gave some attention to the preparation of *N*-phytyl-THII (113).

The introduction of the phytyl group poses problems due to the presence of the

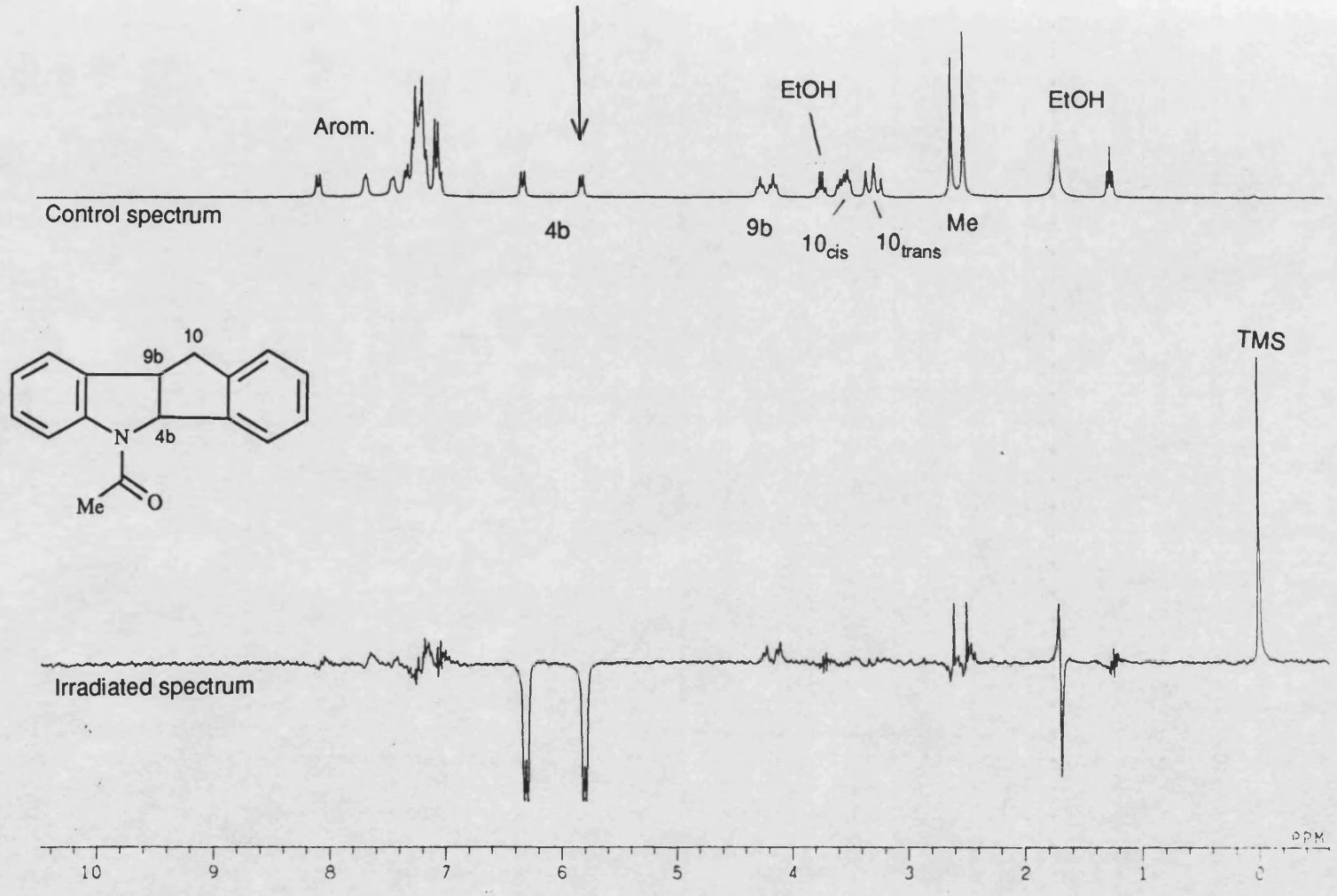
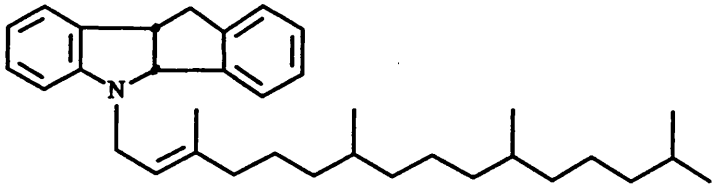
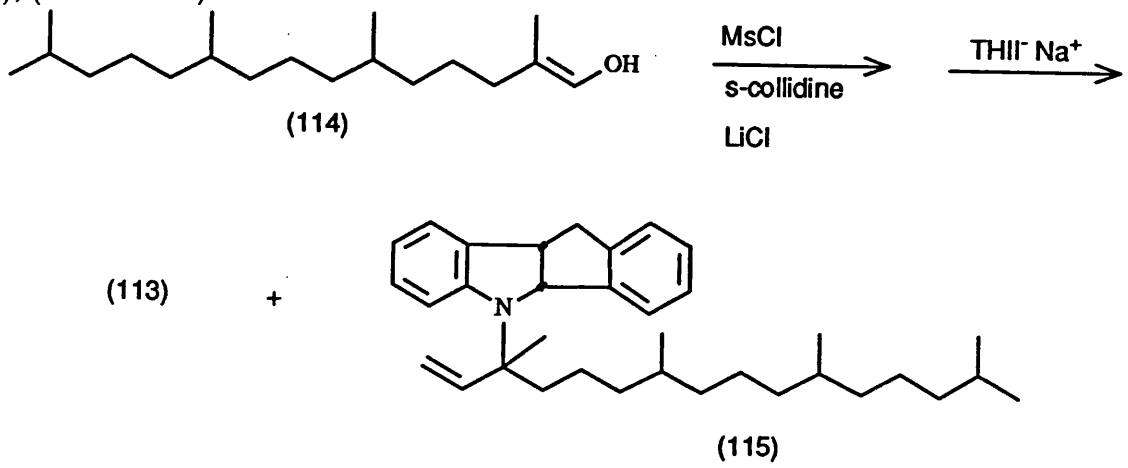

Arom.
EtOH
EtOH
Control spectrum
4b
9b
10cis
10trans
Me
10
9b
N
4b
Me
O
TMS
Irradiated spectrum
ppm
10
9
8
7
6
5
4
3
2
1
0

Figure 3:1



(113)

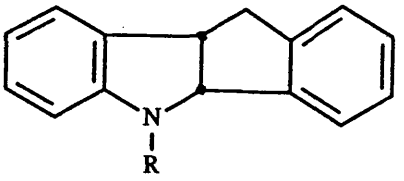
allylic double bond. Phytol (114) can be converted to phytyl chloride (69) using a method described by Meyers and Collington[44] which is known to convert allylic alcohols to allylic halides without allylic rearrangement. The procedure involves the chloride ion displacement of the mesylate group, with the sulphonate ester formed *in situ* from the alcohol and mesyl chloride. 2,4,6–Collidine is employed as base in this reaction. The author used this procedure to obtain a solution of phytyl chloride which was added to a solution of the conjugate base of THII formed using sodium hydride as base. After the usual work–up, t.l.c. analysis indicated the presence of two products from the alkylation reaction, with very similar R_f values {0.75 and 0.80 [EtOAc/petrol(60–80)]}. These were collected together, and analysed by ^1H nmr spectrometry to show that they were a mixture of alkylated THII (113), and the product of alkylation with allylic rearrangement (115), (scheme 3:22).



Scheme 3:22

This problem may have been relieved by isolating the mesylated phytol, and using this as the alkylating agent, however since we wished to have a quick result for the lipophilicity assessment, we decided instead to look at other groups which did not present this

difficulty, but still had the same overall property. These were selected as the straight chained 18–C alkyl and acyl compounds (116) and (117).



(116) R = $C_{18}H_{37}$

(117) R = $COC_{17}H_{35}$

An initial experiment was the attempted alkylation of THII with bromooctadecane, using sodium hydride as base. However we failed to isolate the required product. This result could be due to the C_{18} alkyl chain "wrapping" itself around the alkyl bromide so that its approach to the anion is rendered difficult. We overcame this by improving the nucleophilicity of the anion by using *n*–butyllithium as base in the presence of tetramethylethylenediamine (TMEDA) as a lithium complexing agent. Using this system, it was possible to alkylate THII with bromooctadecane, or acylate it with stearoyl chloride, in 85% and 42% yields respectively. The acylated product was synthesised in response to news from Shertzer in the US that although *N*–acetyl–THII (112) is not a good compound in the cell–free antioxidation assay, it was showing some interesting properties in other related tests.

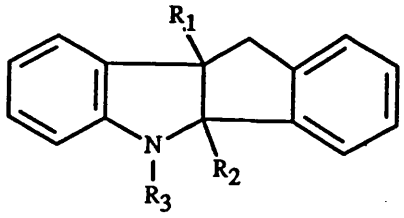
We now know that the activities of these two substrates in the cell free oxidation assay are poor. This could again be due to the steric influence of the hydrocarbon chain which inhibits the approach of an electron transfer species.

After this failure, we considered using other less flexible lipophilic groups, but at this time, Shertzer provided us with partition data (see chapter 4) which suggests that the simple THII compounds are quite lipophilic enough. Shertzer argues that to attempt to increase the fat solubility of the compounds would be counter productive. We are not entirely convinced by this argument, and do not believe that THII is already sufficiently lipophilic to exert optimal activity as a cell intact antioxidant, and remain optimistic that in experiments in animals the hydrocarbon chain may act as a useful directing agent

into the intact cell membrane. We also do not understand the significance of the results on the *N*-acetylated compound, unless it undergoes simple deacetylation *in vivo*. As previously stated, we did not expect this compound to exhibit any significant activity in either cell free, or cell intact test systems.

3:4:4 *Substitution of methyl groups for hydrogen on THII*

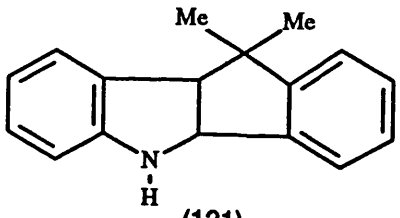
To investigate further the fate of THII on oxidation, a range of compounds where the non-aromatic hydrogen atoms were replaced with methyl groups was prepared. Some obvious targets are; 9b-methyl- (118), 4b,9b-dimethyl- (119), 4b,5,9b-trimethyl- (120), and 10,10-dimethyl-THII (121).



(118) $R_1 = Me, R_2 = R_3 = H$

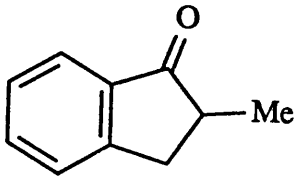
(119) $R_1 = R_2 = Me, R_3 = H$

(120) $R_1 = R_2 = R_3 = Me$

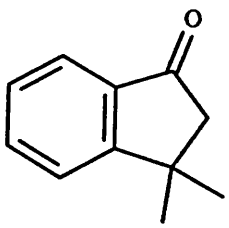


(121)

To make these four compounds utilising the Fisher indole synthesis, two indanones; 2-methyl- (122), and 3,3-dimethyl-1-indanone (123), were required.



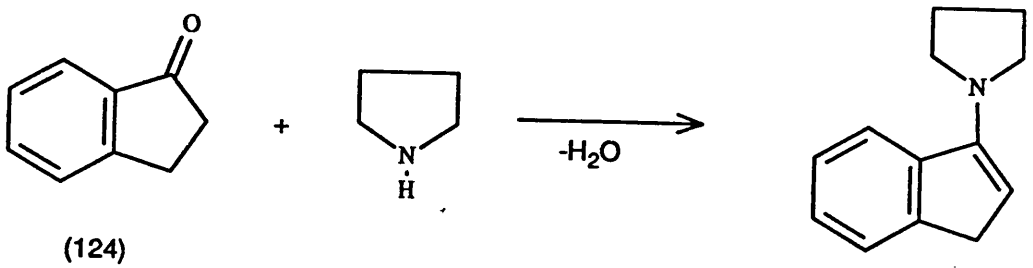
(122)



(123)

Neither of these ketones is commercially available, and so a preparation of each was necessary.

One of the best ways to α–monoalkylate a ketone is *via* enamine chemistry.[45] A ketone such as 1–indanone (124) reacts with a secondary amine such as pyrrolidine to form an enamine (125), (scheme 3:23).



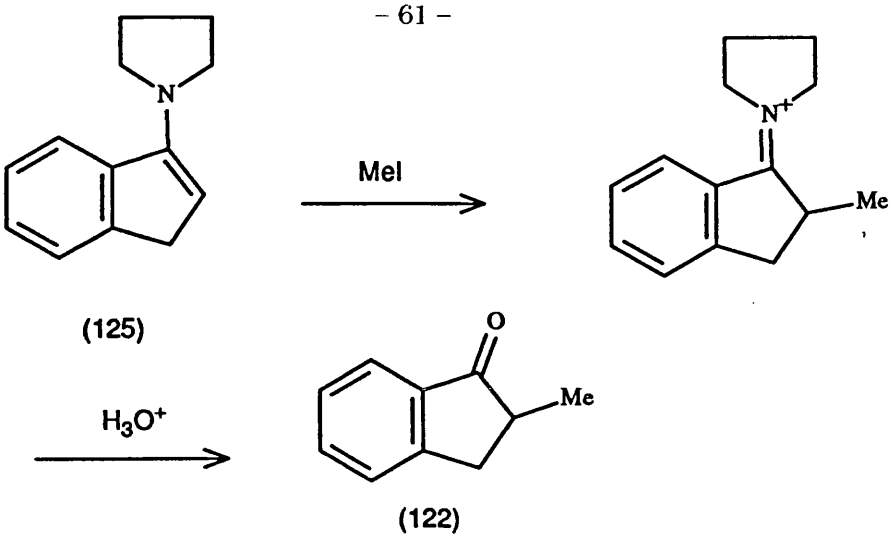
(124) (125)

Scheme 3:23

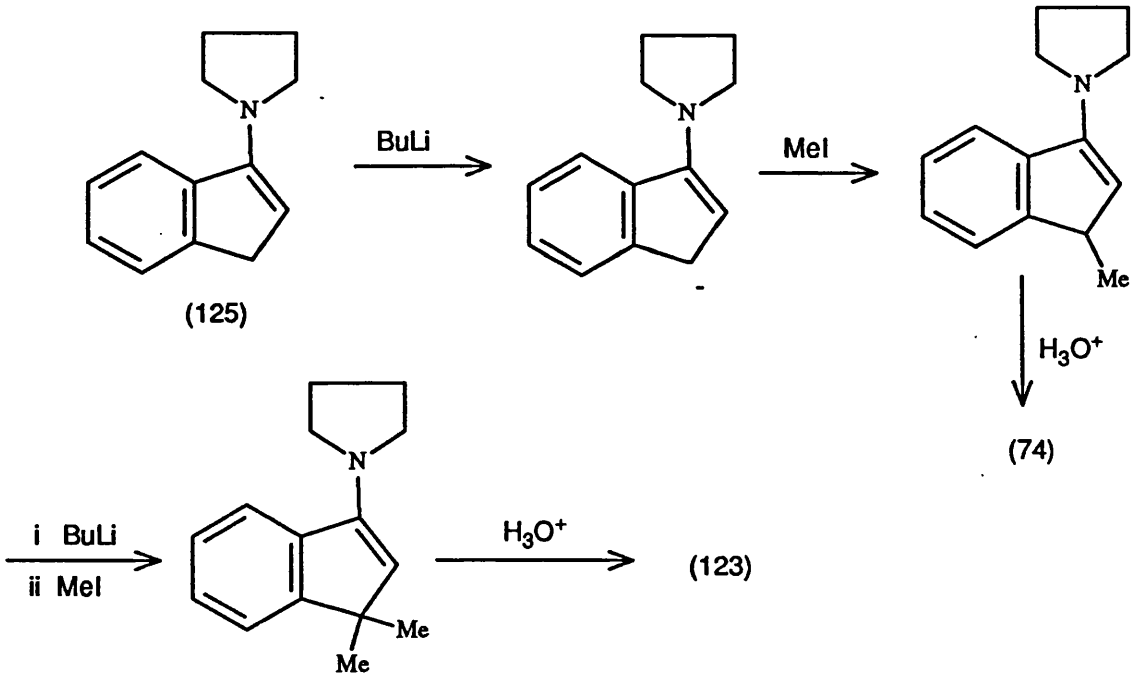
The formation of enamines may be promoted by the addition of an acid catalyst, commonly *p*–toluenesulphonic acid in benzene or toluene solution. The reaction reaches an equilibrium between the enamine, and the free ketone, and is forced to completion by the removal of the water by azeotropic distillation or by the addition of dehydrating agents or molecular sieves. None of these alternative techniques have any real advantage over azeotropic distillation, except in cases when the products are heat sensitive. For 1–indanone, azeotropic distillation is most satisfactory.[46]

Electrophilic alkylation of the pyrrolidine enamine of 1–indanone (125) with iodomethane occurs preferentially at carbon, and hydrolysis of the imine salt thus formed, leads predominantly to the mono–alkylated product (122), (scheme 3:24).

Thompson and Huegi have shown[47] that on treatment of the enamine (125) with butyllithium at –65°C, deprotonation occurs at the β–allylic position (scheme 3:25), alkylation and hydrolysis giving 3–methyl–1–indanone (74). Moreover, the 3–methylenamine may be alkylated further by treatment with a second equivalent of butyllithium in refluxing THF, followed by reaction with iodomethane. Hydrolysis gives 3,3–dimethyl–1–indanone (123) in good yield.



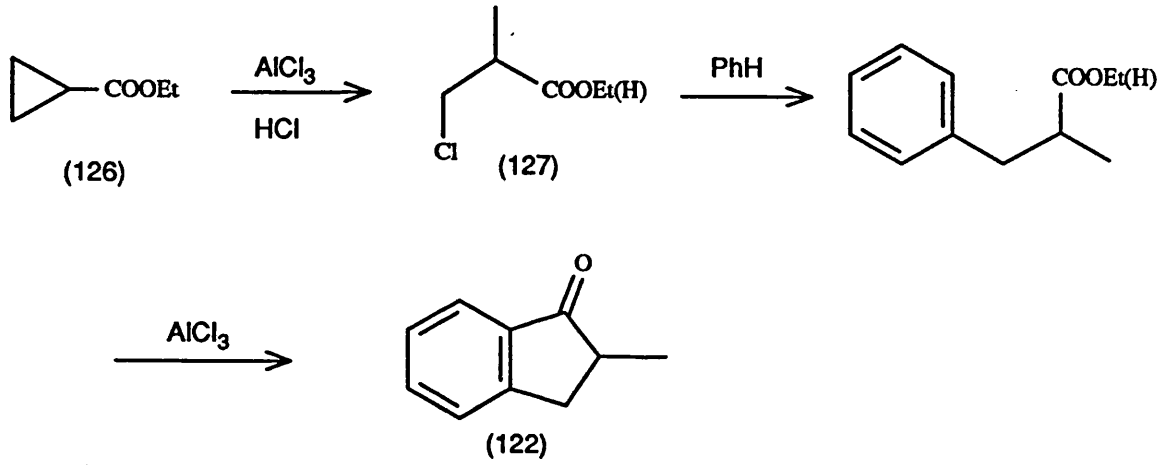
Scheme 3:24



Scheme 3:25

Therefore both of the required indanones (122) and (123) could be conveniently obtained from 1-indanone *via* enamine chemistry. However, enamines are unstable oils which are readily hydrolysed in air, and so are relatively awkward to handle, and so whilst the above procedures were being investigated, other routes to the alkylated indanones were sought.

In 1981, Pinnick and co–workers published the results of the reaction between ethyl cyclopropanecarboxylate (126), and benzene, in the presence of an excess of aluminium chloride,[48] which gave 2–methyl–1–indanone (122) in very good yield. The mechanism of the reaction appears to be as follows (scheme 3:26): aluminium chloride, along with a trace of hydrogen chloride opens the cyclopropane ring to give a mixture of ethyl–3–chloro–2–methylpropionate (127), and the corresponding acid. This compound alkylates benzene to give ethyl–1–methyl–2–phenylpropionate which then undergoes intramolecular acylation to afford the ketone (122) in 93% yield.



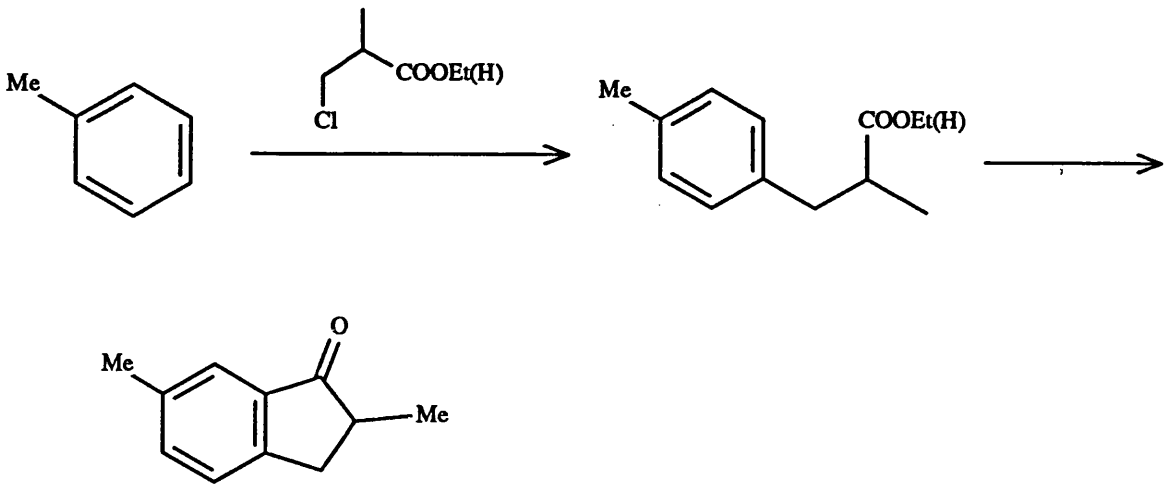
(126) (127) (122)

Scheme 3:26

The authors give additional evidence to prove that the reaction involves alkylation followed by acylation, by repeating it in toluene as solvent and co–reactant. Many products were obtained, but 2,6–dimethyl–1–indanone (128) was isolated in 67% yield, indicating that the initial alkylation is directed principally *para* to the methyl substituent (scheme 3:27).

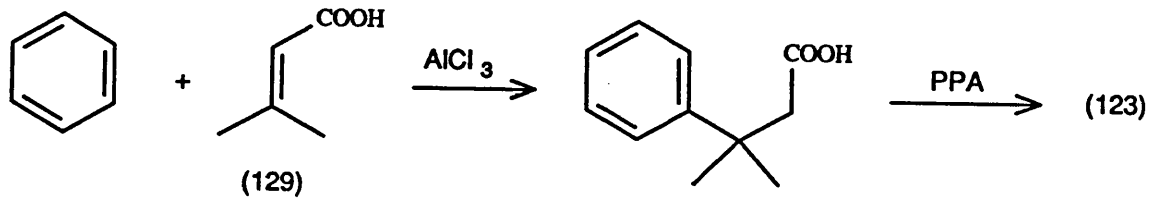
This procedure was used to prepare 2–methyl–1–indanone (122) on a large scale. The product turns brown upon exposure to air and light, and is best stored under an inert atmosphere at –20°C.

3,3–Dimethyl–1–indanone (123) was made in a similar fashion to 3–methyl–1–indanone (74), by the Friedel–Craft alkylation of benzene with dimethylacrylic acid



(128) Scheme 3:27

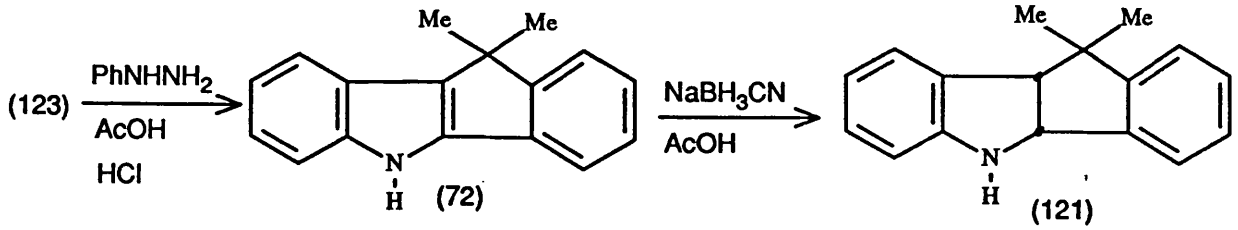
(129) using aluminium chloride as catalyst, followed by intramolecular acylation using PPA as the catalyst, yield 57% (scheme 3:28). This route was found to be preferable for the preparation of a large amount of (78), than the enamine route described earlier which gave poorer yields of a less pure product.



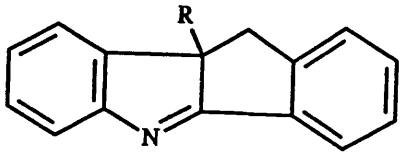
Scheme 3:28

10,10–Dimethyl–5,10–dihydroindeno[1,2–*b*]indole (72) was prepared from 3,3–dimethyl–1–indanone (123), and phenylhydrazine in glacial acetic acid at reflux, using hydrochloric acid as catalyst. The crude tetracyclic product was isolated from the product mixture and crystallised from petrol (60–80°C). Reduction of the indole subunit with sodium cyanoborohydride in glacial acetic acid, was accomplished in quantitative yield (scheme 3:29). Both the indole, and the dihydroindole (121) were assayed for use as antioxidants.

The preparation of the indolenine 9b,10–dihydro–9b–methylindeno[1,2–*b*]indole (130, R = Me), has never been reported, although the homologue 9b–ethyl–9b,10–DHII (130, R = Et) is described in three publications. In two of these, Maeda and Nakazaki

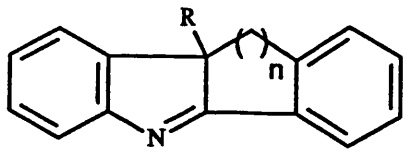


Scheme 3:29



(130)

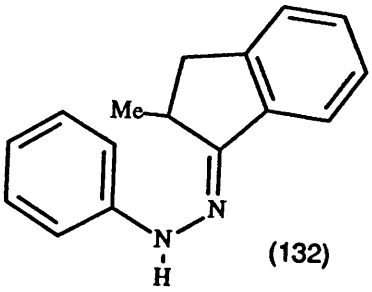
report[49, 50] the preparation of a series of homologues in which the bridge between the indoleno ring, and the benzene ring is increased from one to four methylene groups (131). The motivation for these syntheses is vague, beyond the statement that the physical properties of the products were to be investigated.



(131) R = Me, n = 2-4
R = Et, n = 1

The authors do not comment on any attempt to prepare 9b–methyl–DHII (130, R = Me), or why they chose in the first instance to use an ethyl group as the substituent in (131) when n=1.

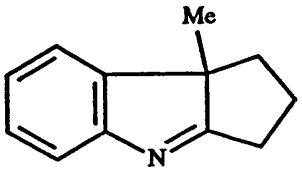
Both Maeda and Nakazaki[50] and Leuchs, in the first of the three papers[51] prepared the indolenine (130, R = Et) by the Fisher route from the hydrazone of 2–ethyl–1–indanone using zinc chloride as catalyst. In both cases, the product obtained from the reaction, was an adduct of the indolenine with zinc chloride, from which the free base was liberated by treatment with dilute ammonia. In our hands, a similar reaction upon the hydrazone of 2–methyl–1–indanone (132), gave only an intractable solid which may be the expected adduct. However, this compound did not respond to treatment with



(132)

ammonia, and we were unable to obtain the required 9b–methyl–DHII (130, R = Me) from it. In view of this difficulty, a non–catalytic Fischer synthesis was investigated as an alternative route to (130) avoiding acids.

Thermal indolisation reactions have been reported in the literature[6] including some work directed towards synthesis of the indolenine 1,2,3,8b–tetrahydro–8b–methylcyclopent[*b*]indole (133).



(133)

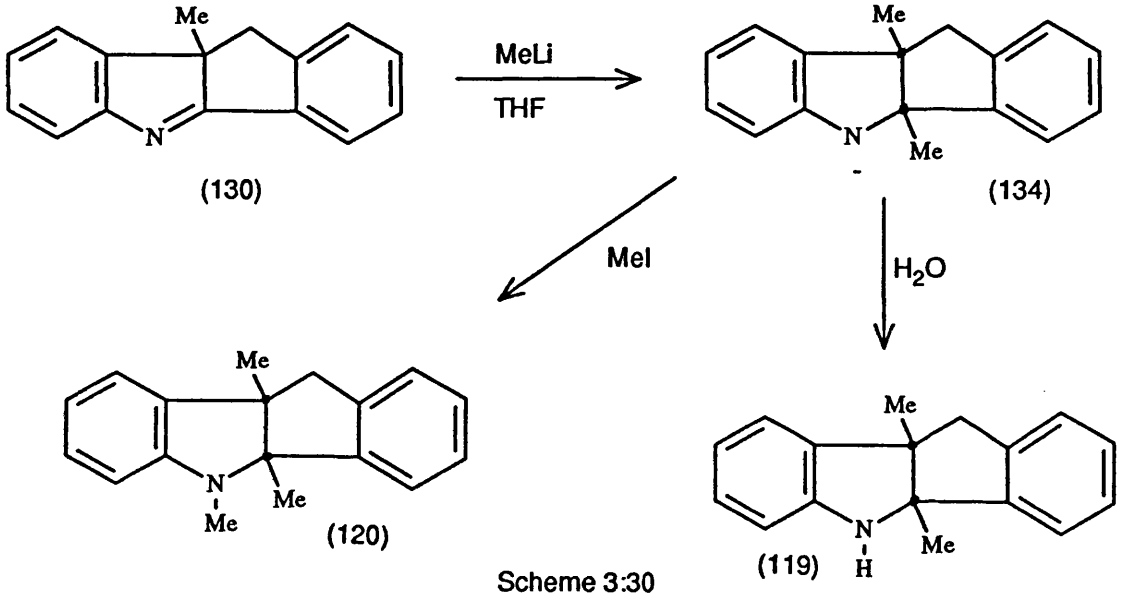
One of the sets of published conditions,[52] involves heating the hydrazone in diethylene glycol (bp 245°C) up to reflux temperature. On repeating this experiment with the hydrazone (132), we noticed that ammonia evolved from the condenser. When this emission ceased, the reaction was cooled and worked–up, and found to contain a number of products. The basic components were isolated, and separated into two fractions by column chromatography. The least polar of these was found to consist of the reduced compound 9b–methyl–THII (118) (45% yield). The more polar compound was also collected, and this demonstrated spectral properties similar to those expected for the indolenine, (11% yield) (this conclusion was confirmed by comparison with an unambiguous sample prepared later).

The low productivity of this reaction was disappointing, and it was obvious that we

required milder conditions to synthesise the indolenine. Fortunately, in 1988, a group of Spanish workers published details of the cyclisation of hydrazones using phosphorus trichloride as catalyst, in DCM at room temperature. [53] These mild conditions, and the fact that as a much weaker Lewis acid than zinc chloride, phosphorus trichloride would be less likely to form complexes with the indolenine, led us to attempt to form (130, R = Me) using this route.

The reaction was relatively successful when performed on the hydrazone (132). The indolenine was obtained in 30% yield (purified product). It was generally used after only a small degree of purification by column chromatography, as the products of subsequent reactions on the compound were easier to isolate. However, a pure sample could be obtained by bulb to bulb distillation of the crude product, although it is not a very stable compound, and needs to be stored in the freezer under an atmosphere of nitrogen.

The indolenine was converted into the 4b–methyl anion (134), by reaction with methyllithium at -78°C, and quenched either with water to form (119), or with iodomethane to form (120), (scheme 3:30).



Scheme 3:30

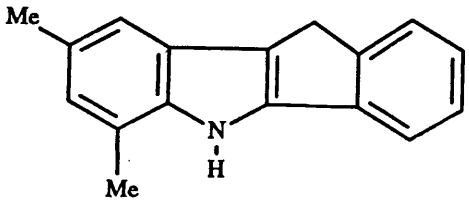
NOe experiments performed on the dimethyl–THII (~~74~~ 134) indicated that it maintains the expected *cis* relationship between the two methyl groups, thus a mutual

enhancement of *ca.* 15% was observed between the proton resonances of each group.

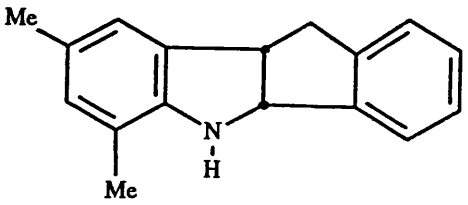
All the THII derivatives thus prepared, were sent to the US for pharmacological assessment. However during transit, 4b,5,9b–trimethyl–THII (120), which was contained in a sealed vial, became blue in colour. Since it had left us as a colourless solid, the vial was promptly returned unopened to us by Shertzer. Some comments on the origin of this colour are made in section 4:2:3.

3:4:5 *6,8-Dimethyl-DHII and -THII*

6,8–Dimethyl–DHII (135) was synthesised in moderate yield by the usual procedure from 2,4–dimethyl PHENYL hydrazine hydrochloride, and 1–indanone, in glacial acetic acid. The purification procedure needed to obtain a pure product was however quite laboured as the reaction does not go as cleanly as do other Fisher reactions in the series. Reduction to the indoline, however, was accomplished readily using standard conditions to give 6,8–dimethyl–THII (136) as a colourless solid. This compound, in fact, turned out to be very active as an antioxidant.



(135)

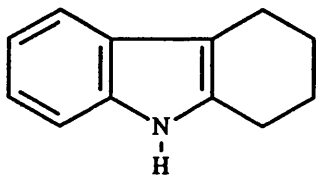


(136)

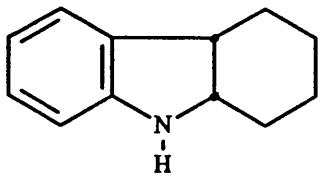
3:4:6 *Additional Compounds*

A number of compounds were assayed by Shertzer in Cinncinnatti as controls. Most of these were available from standard sources (Aldrich), but a few were provided by this laboratory. These are; 1,2,3,4–tetrahydrocarbazole (28), readily available from one of the undergraduate practical classes, and *cis*–1,2,3,4,4a,9b–hexahydrocarbazole (137) which can be obtained from it by reduction with sodium cyanoborohydride (98%

yield).



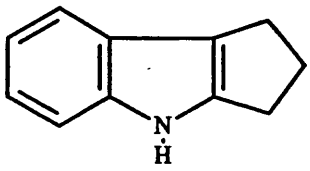
(28)



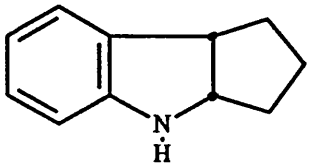
(137)

This compound has been prepared in both *cis* and *trans* forms by Perkin and Plant. After reducing 1,2,3,4–tetrahydrocarbazole (28) with zinc and hydrochloric acid and isolating the major product which was considered to be the *cis* isomer,[54] they repeated the reaction using 1200g of indole, and after working through a long series of purifications, eventually obtained 12g of the *trans* isomer.[55] This compound has a melting point 28°C higher than the *cis*. Reduction with sodium cyanoborohydride used in our work also gave the *cis* reduction product, none of the *trans* isomer was detected.

Also prepared were the documented 1,2,3,4–tetrahydrocyclopent[*b*]indole, (138) from cyclopentanone using a method described by Perkin and Plant in 1923,[56] and its reduction product *cis*–1,2,3,3a,4,8b–hexahydrocyclopent[*b*]indole (139), a compound first described by Plant and Rippon in 1928.[57]



(138)

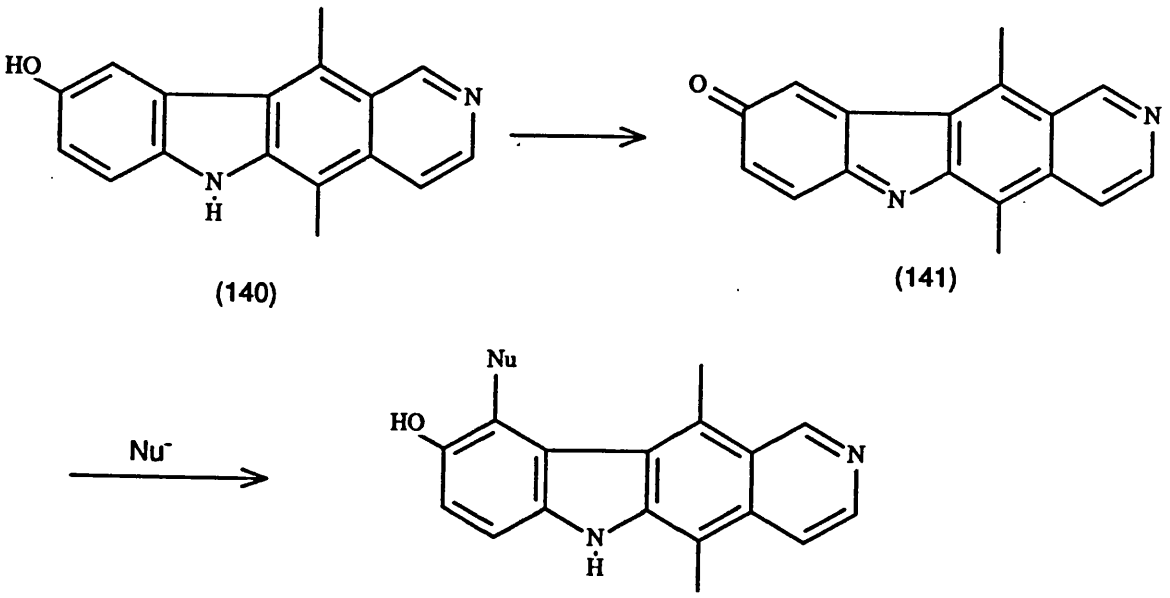


(139)

These four indoles showed good antioxidative properties, but did not exhibit the levels of activity noted for the reduced indenoindoles.

The biological assays show that in the DHII series, the most potent compound was (at that time) 8–methoxy–DHII, and this may indicate that the radical cation/radical formed from it by one electron oxidation/and deprotonation of the nitrogen, is stabilised by conjugation with the *para*–methoxy group. It may seem that by analogy with vitamin

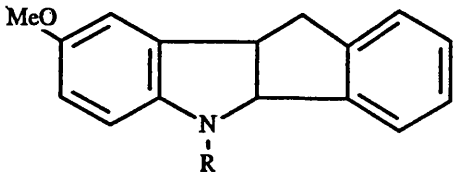
E, an obvious target should be the corresponding 8–hydroxy compound. Certainly, in other series such as the pyrido[3,4–*b*]carbazoles, oxidation of 9–hydroxyellipticine (140) leads to the iminoquinone (141). This compound is cytotoxic, and reacts *in ,vivo* with available bio–nucleophiles [58] (scheme 3:31). However, this reaction involves the formation of the phenoxy radical rather that a nitrogen radical, and for this reason, we have not proceeded with the preparation of 8–hydroxy–DHII or –THII.



(140) (141)

Scheme 3:31

However, we did decide to synthesise the THII analogue of the 8–methoxy–DHII (142), and also the *N*–methyl derivative (143). This was achieved by the standard methods described above.



(142) R = H

(143) R = Me

References to chapter 3

1. I. T. Hogan, *Ph.D. Thesis (University of Bath)*, 1985.
2. J. Hausmann, *Chem. Ber.*, 1889, **22**, 2019.
3. F. S. Kipping, *J. Chem. Soc.*, 1894, **65**, 480.
4. H. Leuchs and G. Kowalski, *Chem. Ber.*, 1925, **58**, 2822.
5. B. Robinson, *Chem. Rev.*, 1963, **63**, 373.
6. B. Robinson, *Chem. Rev.*, 1969, **69**, 227.
7. B. Robinson, *The Fischer Indole Synthesis,* Wiley (1983).
8. W. Carruthers and N. Evans, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1523.
9. T. Itahara and T. Sakakibara, *Synthesis*, 1978, 607.
10. K. Bill, G. G. Black, C. P. Falshaw, and M. Sainsbury, *Heterocycles*, 1983, **20**, 2433.
11. G. G. Black, *Ph.D. Thesis (University of Bath)*, 1985.
12. V. Kanaoka, Y. Ban, K. Miyashnita, K. Irie, and O. Yonemitsu, *Chem. Ind. (London)*, 1965 , **11**, 473.
13. V. Kanaoka, Y. Ban, K. Miyashnita, K. Irie, and O. Yonemitsu, *Chem. Pharm. Bull.*, 1966, **14**, 934.
14. N. N. Suvorov, M. Ya. Bykhovskii, L. I. Dmitrevskaya, N. E. Starostenko, Yu. I. Smushkevich, and N. N. Shkilkova, *Tr. - Mosk. Khim. - Tekhnol. Inst. Im. D. I. Medeleeva*, 1977, **94**, 46. C. A. 1980 R110078
15. J. J. Eisch and T. Abraham, *Tetrahedron Lett.*, 1976, **20**, 1647.
16. D. L. Horrocks and H. O. Wirth, *J. Chem. Phys.*, 1967, **47**, 3241.
17. D. L. Horrocks and H. O. Wirth, *J. Chem. Phys.*, 1968, **49**, 2907.

18. D. L. Horrocks, *J. Chem. Phys.*, 1968, **49**, 2913.

19. D. L. Horrocks and H. O. Wirth, *U. S. Patent*, 1969, 3,478,208. C. A. 1970 p43438c

20. S. A. Bryant and S. G. P. Plant, *J. Chem. Soc.*, 1931, 93.

21. S. G. P. Plant and M. L. Tomlinson, *J. Chem. Soc.*, 1931, 3324.

22. W. Bonthone and D. H. Reid, *J. Chem. Soc.*, 1959, 2773.

23. V. G. Kempter, M. Schwalba, W. Stoss, and K. Walter, *J. Prakt. Chem.*, 1962, **18**, 39.

24. T. Abraham and D. Curran, *Tetrahedron*, 1982, **38**, 1019.

25. T. Abraham, *Monatsh. Chem.*, 1982, **113**, 1275.

26. R. M. Letcher, K.-W. Ng, and K.-K. Cheung, *J. Chem. Soc., Chem. Commun*, 1987, 1602.

27. A. G. Perkin and F. Thomas, *J. Chem. Soc.*, 1909, **95**, 793.

28. B. Witkop, *Liebigs Ann. Chem.*, 1947, **558**, 98.

29. I. Tabushi and K. Morimitsu, *Tetrahedron Lett.*, 1986, **27**, 51.

30. W. Bhatti, M. Bhatti, P. Imber, A. Lee, and B. Lorenzen, *J. Pharm. Sci.*, 1972, **61**, 307.

31. A. D. Adler, F. R. Longo, and F. Kampas, *J. Inorg. Nucl. Chem.*, 1970, **A13**, 727.

32. J. M. Anderson and J. K. Kochi, *J. Am. Chem. Soc.*, 1970, **92**, 1651.

33. F. Minisci, *Synthesis*, 1972, 1.

34. O. Hammerich, N. S. Moe, and V. D. Parker, *J. Chem. Soc., Chem. Commun.*, 1972, 156.

35. Y. Kumar and L. Florvall, *Synth. Commun.*, 1983, **13**, 489.

36. E. Niki, Y. Yamamott, and Y. Kamiya, *Life Chemistry Reports*, 1985, **3**, 35.

37. A. G. Anastassiou, H. S. Kasma, and D. Hauger, *J. Chem. Soc., Chem. Commun.*, 1981, 647.

38. Y.-C. Kong, K. F. Cheng, K.-H. Ng, P. P.-H. But, Qian-Li, S.-X. Yu, H.-T. Chang, R. C. Cambie, T. Kinoshita, W. S. Can, and P. G. Waterman, *Biochem. Syst.*, 1986, **14**, 491. and references therein

39. K.-F. Cheng, T.-T. Chan, T.-F. Lai, and Y.-C. Kong, *J. Chem. Soc., Perkin Trans. I*, 1988, 3317. (Synthesis)

40. J. Bergman and L. Venemalm, *Tetrahedron Lett.*, 1988, **29**, 2993. (Synthesis)

41. S. R. Cawley and S. G. P. Plant, *J. Chem. Soc.*, 1922, **121**, 827.

42. J. W. Armit and R. Robinson, *J. Chem. Soc.*, 1922, **121**, 1562.

43. S. G. P. Plant and N. M. Beyts, *J. Chem. Soc.*, 1939, 1534.

44. A. I. Meyers and E. W. Collington, *J. Org. Chem.*, 1971, **36**, 3044.

45. J. K. Whitesell and M. A. Whitesell, *Synthesis*, 1983, 517.

46. E. D. Bergmann and E. Hoffmann, *J. Org. Chem.*, 1961, **26**, 3555.

47. H. W. Thompson and B. S. Huegi, *J. Chem. Soc.; Perkin Trans. I*, 1976, 1603.

48. H. W. Pinnick, S. P. Brown, E. A. Mclean, and L. W. Zoller, *J. Org. Chem.*, 1981, **46**, 3758.

49. M. Maeda and N. Nakazaki, *Chem. Ind. (London)*, 1960, 719.

50. M. Maeda and N. Nakazaki, *Bull. Chem. Soc. Japan*, 1962, **35**, 1380.

51. H. Leuchs, *Liebigs Ann. Chem.*, 1928, **461**, 27.

52. A. H. Kelly, D. H. McLean, and J. Parrick, *Can. J. Chem.*, 1965, **43**, 296.

53. G. Baccolini, R. Dapozzo, and P. C. Todesco, *J. Chem. Soc., Perkin Trans. I*, 1988, 971.

54. W. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, 1924, **125.**

55. J. Gurney, W. H. Perkin, and S. G. P. Plant, *J. Chem. Soc.*, 1927, 2676.

56. W. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, 1923, 3242.

57. S. G. P. Plant and D. M. L. Rippon, *J. Chem. Soc.*, 1928, 1906.

58. S. Archer, B. S. Ross, L. Pica–Mattoccia, and D. Cioli, *J. Med. Chem.*, 1987, **30**, 1204.

A1 Appendix 1, structure of indeno[1,2-*b*]indoles

A1:1 *Nmr assignment of DHII*

In 1987, a review appeared in the literature documenting the ^{13}C spectra of 298 indole compounds unsubstituted in the benzene ring.[1] The review does not contain the data for DHII (20) or any similar molecules, but some of the published data seems to correlate with the assignments that we have made (see table A1:1). These were made unambiguously using modern nmr techniques as described below, making DHII one of the few compounds containing the indole nucleøus that has been fully characterised.

DHII bears many features which render this sort of work relatively easy to undertake. The compound is easy to prepare in a large scale, and in a high degree of purity. Unfortunately it is not soluble in many organic solvents, and so the nmr experiments had to be undertaken in deuterated DMSO. In both the proton and the carbon–13 spectra, most of the nuclei resonate within small ranges; in the proton spectra, eight out of the eleven hydrogen atoms resonate between 7 and 7.7ppm (w.r.t. TMS), and in the carbon spectrum, all but one of the nuclei resonate at between 110 and 150ppm. This implies that for the less sensitive experiments, smaller spectral windows could be set (accounting for the fold back of extraneous signals), thus enhancing the resolution, and decreasing the accumulation time.

The 1D spectra yield little information on assignments except those due to C–10, or the one due to the N–H resonance. A COSY–45 experiment was used to assign the eight aromatic protons into two groups of four corresponding to each benzene ring (figure A1:1), and these were assigned with the help of a single 1D nOe difference experiment. In this, we irradiated the resonance at 11.5ppm due to the N–H proton, and detected a nOe to two of the signals within the aromatic

Table A1:1

Nmr assignments for DHII		
	Resonance (DMSO) w.r.t. TMS	
position	δ_C	δ_H
1	125.6	7.51
2	124.7	7.20
3	126.8	7.36
4	117.9	7.67
4a	135.3	–
4b	143.7	–
5	–	11.6
5a	140.9	–
6	112.6	7.52
7	121.6	7.14
8	119.5	7.07
9	118.7	7.57
9a	124.3	–
9b	120.0	–
10	30.0	3.67
10a	147.8	–

envelope (a nOe was also detected to the signal due to C–10, in fact irradiating at C–10 led to a small enhancement of all the other protons in the spectrum), in an approximate ratio of 2:3 (C–4:C–6). In figure A1:1, the connections from C–1 through to C–4 are mapped out as an example of these assignments.

The appropriate carbon resonances were assigned to the protons by use of a C–H correlation experiment (figure A1:2), but this gave – as expected – no

information on the assignment of the quaternary centres; this was achieved with the aid of a carbon–carbon INADEQUATE experiment.

In order to overcome the insensitivity of the responses examined in such an experiment, the sample was made up as follows: 250mg of DHII was used in $0.3cm^3$ of DMSO, to this was added *ca* 25mg of chromium(III) acetylacetonate. This paramagnetic compound has the effect of diminishing the relaxation time of the signals – this causes the proton spectrum to be broadened – but we were able as a result, to reduce the pulse delay between respective accumulations from 5–6 seconds down to 0.8 seconds. The time delay between the pulses in the sequence used to generate the double quantum coherences was set according to a coupling constant for adjacent aromatic carbon atoms of 55Hz. 32 slices consisting of 16000 points each were collected with about 3000 accumulations per slice. This gave a total accumulation time of about 56 hours. Details of the resulting spectrum are shown in figure A1:3. Using this plot, we were able to assign all of the carbon signals in DHII thus showing the great potential of the 2D INADEQUATE as a tool for proving chemical structure assuming relatively unlimited supplies of the desired compound.

In figure A1:3, the diagonal line represents the centre of gravity upon which all the connections made must have their midpoints. A selection of connections between the INADEQUATE responses are added to show how the assignments are made with respect to the quaternary carbon atoms. The vertical connections link responses due to the same signal.

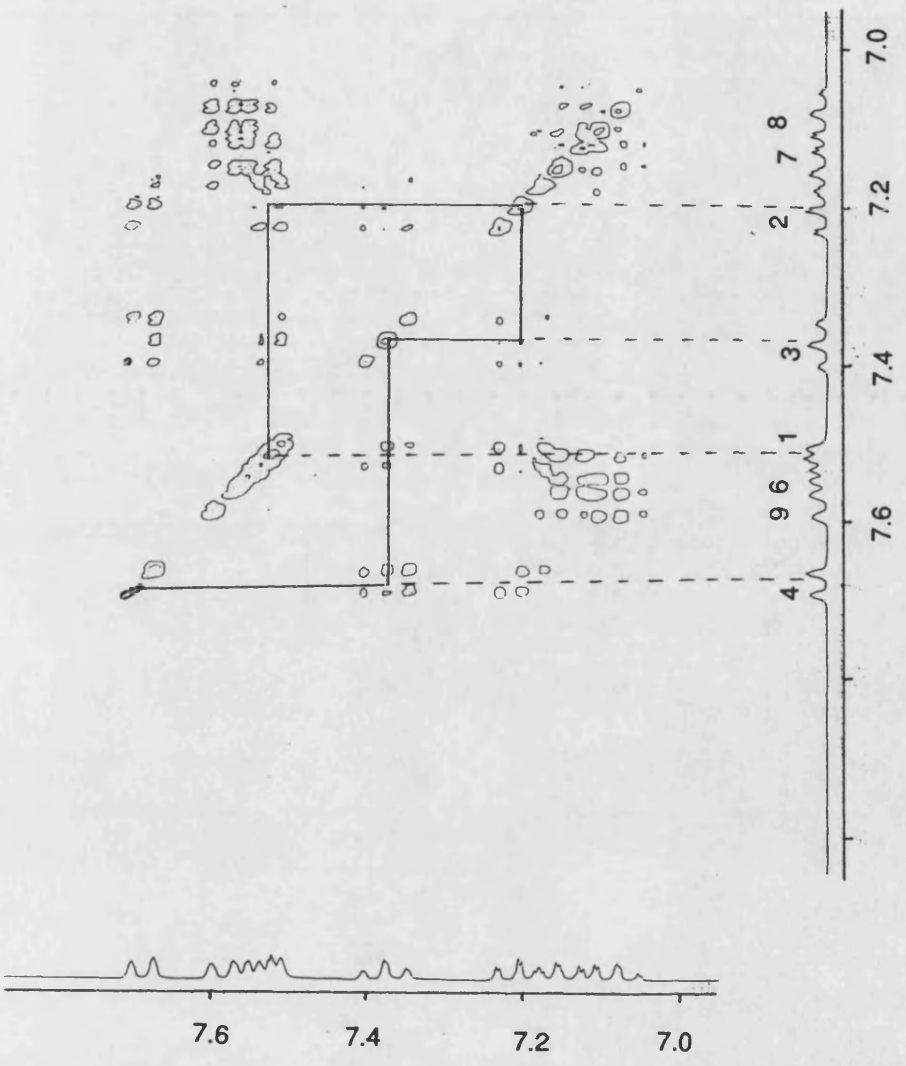
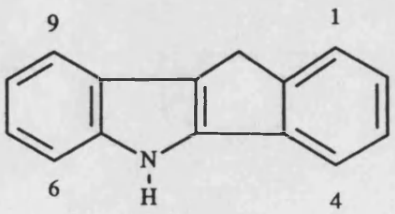


Figure A1:1

COSY-45 of DHII (20)



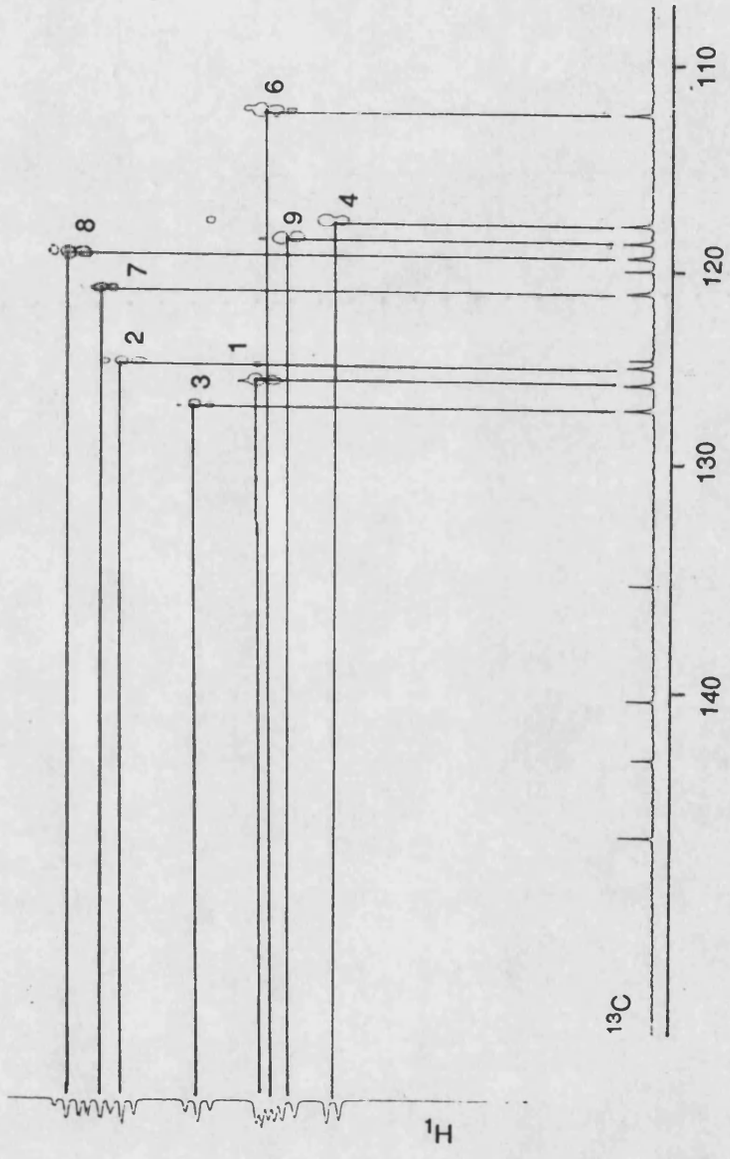


Figure A1:2

C-H correlation of DHII

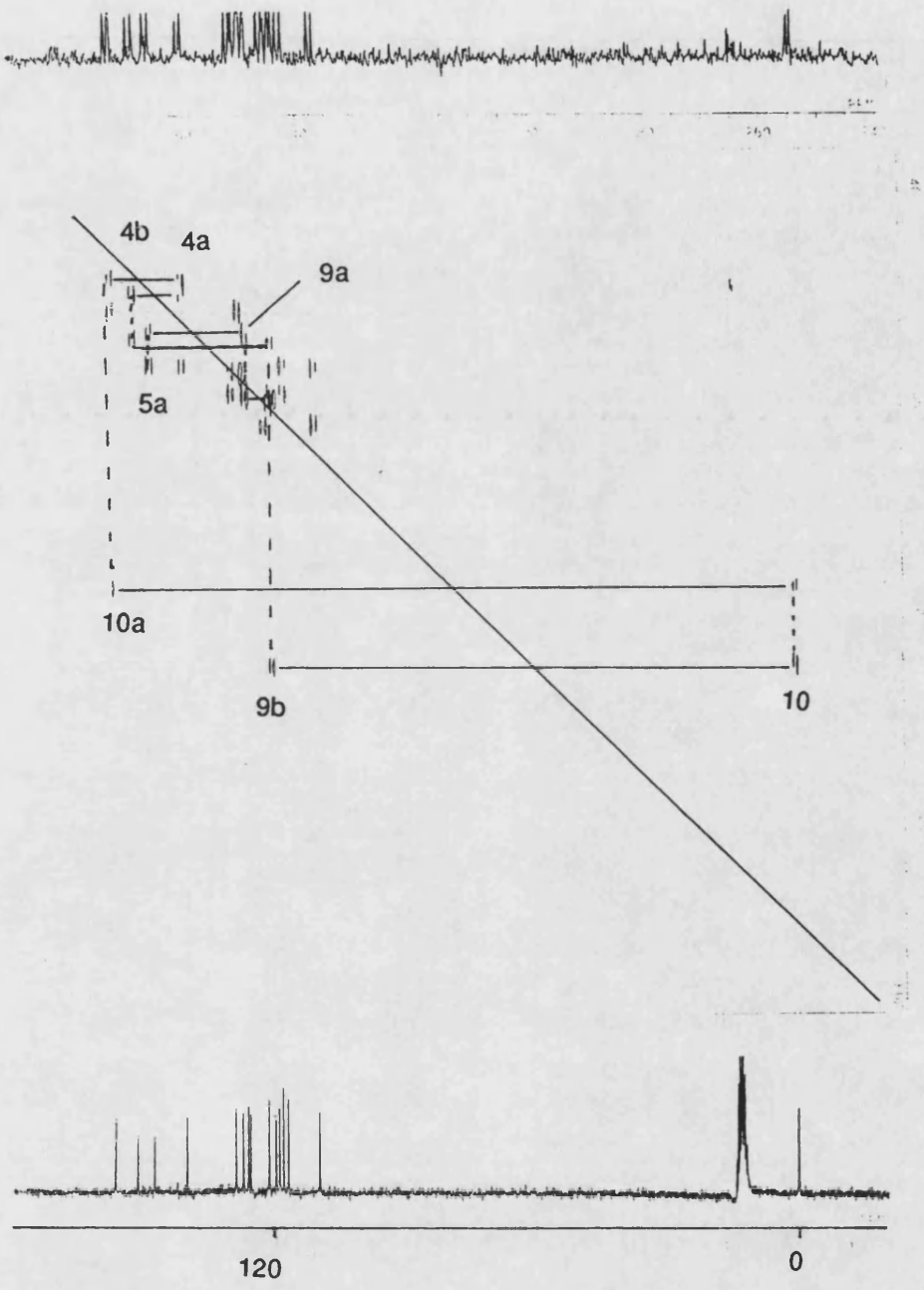
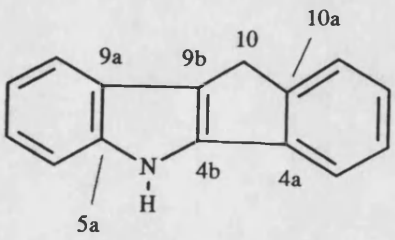


Figure A1:3

2D INADEQUATE of DHII



A1:2 Structure of THII

Although it seemed likely that the sodium cyanoborohydride reduction of DHII would lead to *cis* addition on THII (100), we required evidence that this was indeed so. This evidence came in two ways; *via* an investigation of the coupling constants in the ^1H spectrum and comparison with theoretical calculations of these constants, and from a single crystal X–ray experiment.

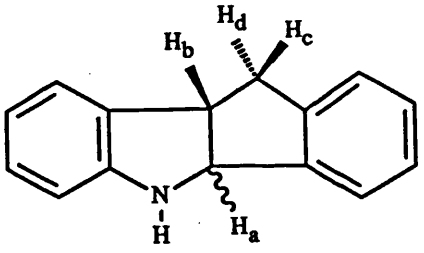
A1:2:1 *Molecular modeling of THII*

A model of both possible conformations of THII was built up onto an Evans and Sutherland terminal using the interactive molecular graphics package INSIGHT (BIOSYM Technologies, San Diego). Each model was minimised using DISCOVER (BIOSYM Technologies) a molecular modelling program using a modified Newton algorithm. The program used a DISCOVER type force–field[2] containing no cross terms, or electrostatic interactions, but using a parabolic bond–stretch term. The minimised structures were examined using INSIGHT, and the three dihedral angles measured – these are listed in table A1:1. Theoretical coupling constants were calculated using the Karplus equation[3] and these compared with the experimental constants as measured from the nmr spectrum.

As can be seen in table A1:2, the best match for the theoretical and experimental data, is made for the compound with *cis* geometry.

Table A1:2

Vicinal coupling constants of THII					
Coupled protons	Theoretical				Experimental
	dihedral angle		$^3J_{calc.}$		$^3J_{expt.}$
	cis	*trans*	*cis*	*trans*	Hz.
H_a–H_b	1.2°	-175.5°	8.2	9.2	8.4
H_b–H_c	-0.2°	-44.4°	8.2	4.1	8.4
H_b–H_d	-119.9°	-168.0°	2.1	8.8	2.0



THII

A1:2:2 *X-Ray structure of THII*

A sample of THII was recrystallised slowly from ethyl acetate/petrol to yield colourless, air stable needles suitable for X–ray analysis. The determined structure showed that the molecule was in fact the *cis* isomer (figure A1:4). Table A1:2 lists the atomic co–ordinates and thermal parameters, table A1:3 the bond lengths, and table A1:4 lists the bond angles. The experimental details were as follows:

Crystal data $C_{15}H_{13}N$, M = 207.3, a = 12.785(4), b = 4.961(3), c = 17.210(5)Å, V = 1091.46Å^3 space group $Pn2_1a$, Z = 4, $d_{calc.}$ = 1.261gcm^3, F(000) = 440 λ = 0.71069Å. μ(Mo–K_α) = 0.38cm^{-1}.

Data collection and Processing The data was collected on a Hilger and Watts Y290 automatic 4 circle diffractometer. 833 reflections were measured (range $2 < \theta < 22$) of which 563 were unique and had $I(\sigma) > 3\sigma$. Data were corrected for Lorentz and polarisation effects, but not for absorbtion. The structure was solved by direct methods using SHELX86.[4] Full matrix least squares refinement over 11 cycles with all atoms treated isotropically, hydrogens being included in calculated positions, but not refined, showed final R and R_w values were 7.86 and 8.15% respectively. Since the origin in the space group $Pn2_1a$ is along the 2_1 axis, the y co–ordinate of N_1 was fixed in all least squares refinement cycles.

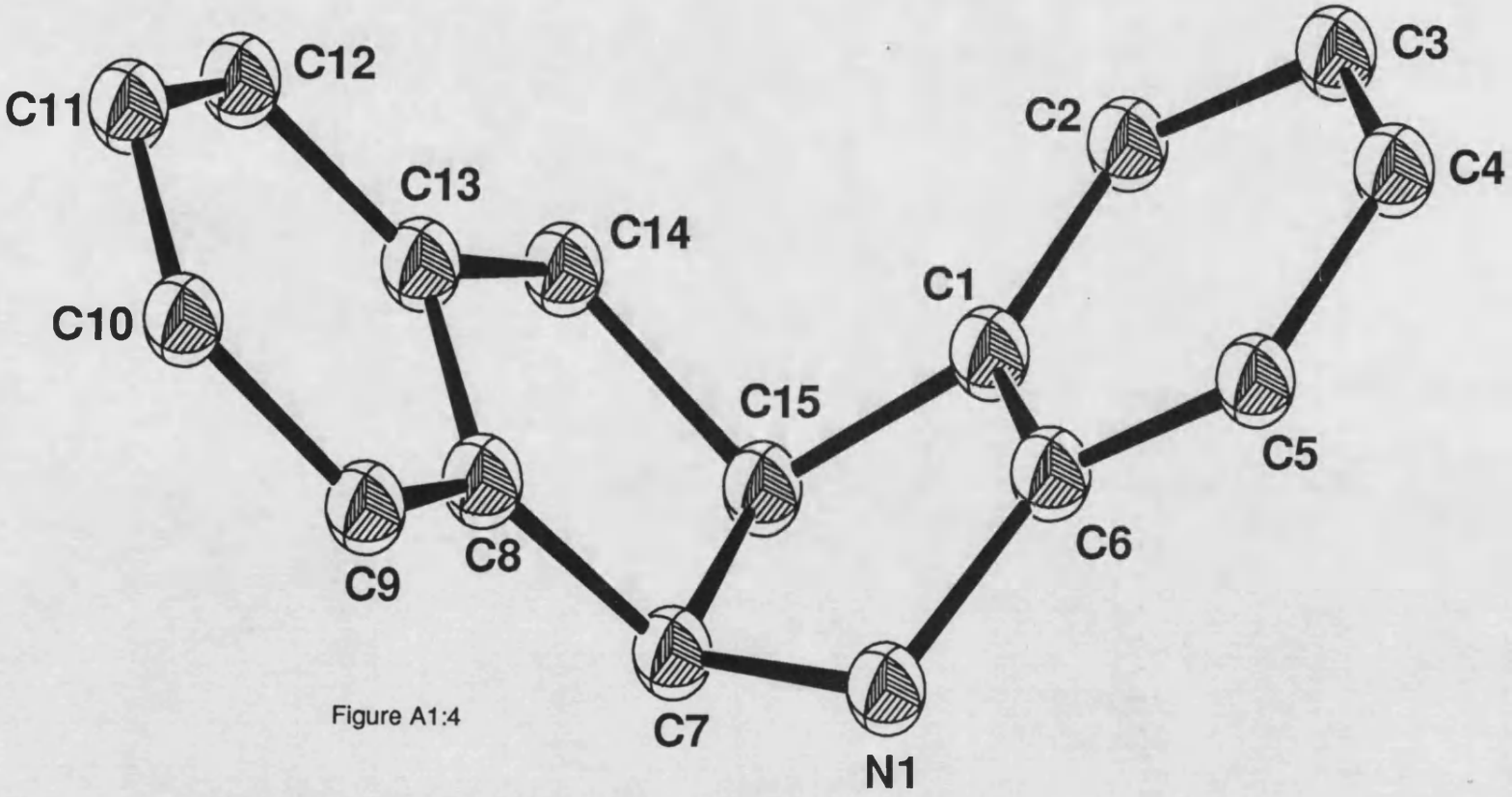


Figure A1:4

ORTEP plot of THII (100)

Table A1:3

Fractional atomic co–ordinates of THII and thermal parameters for THII.				
Atom	x	y	z	U(iso)
N1	0.0699(5)	0.1131	0.4677(4)	0.060(2)
C1	0.2433(6)	0.1116(32)	0.4241(5)	0.058(2)
C2	0.3473(7)	0.1767(32)	0.4268(5)	0.071(3)
C3	0.3830(7)	0.3386(34)	0.4894(5)	0.076(3)
C4	0.3156(7)	0.4266(32)	0.5439(6)	0.076(3)
C5	0.2099(6)	0.3646(31)	0.5418(5)	0.066(3)
C6	0.1753(6)	0.1999(29)	0.4804(5)	0.056(2)
C7	0.0694(6)	0.0118(39)	0.3850(4)	0.052(2)
C8	0.0364(6)	0.2120(27)	0.3267(5)	0.053(2)
C9	–0.0535(7)	0.3701(32)	0.3247(6)	0.069(3)
C10	–0.0688(8)	0.5444(33)	0.2618(6)	0.083(3)
C11	0.0041(7)	0.5716(36)	0.2053(7)	0.087(3)
C12	0.0926(7)	0.4144(35)	0.2060(6)	0.074(3)
C13	0.1103(6)	0.2368(29)	0.2673(5)	0.058(2)
C14	0.1998(6)	0.0556(33)	0.2794(5)	0.064(3)
C15	0.1844(6)	–0.0538(32)	0.3646(4)	0.060(3)

Table A1:4

Bond lengths (Angstroms) for THII			
bond	disance	bond	distance
N1 - C6	1.431(10)	N1 - C7	1.509(11)
C1 - C2	1.369(11)	C1 - C6	1.373(12)
C1 - C15	1.513(13)	C2 - C3	1.419(15)
C3 - C4	1.346(12)	C4 - C5	1.387(12)
C5 - C6	1.407(13)	C7 - C8	1.473(13)
C7 - C15	1.546(11)	C8 - C9	1.392(12)
C8 - C13	1.396(11)	C9 - C10	1.400(15)
C10 - C11	1.353(13)	C11 - C12	1.374(14)
C12 - C13	1.393(14)	C13 - C14	1.471(13)
C14 - C15	1.575(12)		

Table A1:5

Bond angles for THII			
bonds	angle	bond	angle
C7 – N1 – C6	104.4(7)	C6 – C1 – C2	121.1(9)
C15 – C1 – C2	129.4(9)	C15 – C1 – C6	109.6(7)
C3 – C2 – C1	118(1)	C4 – C3 – C2	120(1)
C5 – C4 – C3	122(1)	C6 – C5 – C4	117(1)
C1 – C6 – N1	113.1(8)	C5 – C6 – N1	125.8(8)
C5 – C6 – C1	121.0(8)	C8 – C7 – N1	114.8(9)
C15 – C7 – N1	106.3(6)	C15 – C7 – C8	105.1(7)
C9 – C8 – C7	129.2(8)	C13 – C8 – C7	111.4(7)
C13 – C8 – C9	119.4(9)	C10 – C9 – C8	118.8(9)
C11 – C10 – C9	121(1)	C12 – C11 – C10	120(1)
C13 – C12 – C11	120(1)	C12 – C13 – C8	120.0(8)
C14 – C13 – C8	111.6(8)	C14 – C13 – C12	128.3(8)
C15 – C14 – C13	104.2(7)	C7 – C15 – C1	101.9(8)
C14 – C15 – C1	112.4(9)	C14 – C15 – C7	104.9(7)

References to appendix 1

1. M. S. Morales–Rios, J. Espineira, and P. Joseph–Nathan, *Magn. Reson. Chem.*, 1987, **25**, 377.

2. P. Dauber–Osguthorpe, V. A. Roberts, D. J. Osguthorpe, J. Wolff, M. Genest, and A. T. Hagler, *Proteins: structure, function and genetics.*, 1988, **4**, 31.

3. D. H. Williams and I. Fleming, *Spectroscopic Methods in Organic Chemistry*, , p. 100McGraw Hill (1980).

4. G. M. Sheldrick, *SHELX86 a computer program for crystal structure determination*, University of Gottingen (1986).

4:1 Biochemistry of indenoindoles

The indenoindoles and other structurally related compounds discussed in chapter 3, were packaged and sent to the University of Cincinnati for biological assay by Professor Shertzer and his group – this work is incomplete, and results are still coming through. Additionally, work is more recently being undertaken in Sweden by the pharmaceutical company A. B. Hässle who are also funding the patent application which will cover these compounds.

The indenoindoles exhibit a number of very important properties, which may have direct relevance to several disease indications. Thus the following effects are being evaluated and assessed in an attempt to find a drug candidate:

i) The compounds show antioxidant and radical scavenging ability as evidenced by their inhibition of the iron promoted peroxidation of lipids.

ii) They protect against liver damage in mammals caused by carbon tetrachloride, acetaminophen, or nitrosodimethylamine. (At least one of these chemicals (CCl_4) operates through the participation of radical intermediates[1] (see chapter 2) and so it is likely that a relationship exists between this property and the radical scavenging effect of the indenoindoles.

iii) The indoles also inhibit lipid peroxidation generated by UV light, and yet most of the compounds are themselves unaffected by light. This means that the compounds have potential as skin care products, for example, as sun blockers. In addition, the indenoindoles are expected to protect against tumour promotion in the skin caused by UV irradiation.

iv) As briefly mentioned in section 3:4, the indenoindole compounds stabilise biological membranes – in particular those of red blood cells.

v) More interestingly, recent studies show that THII (100) protects against post-ischaemic reperfusion injury. This injury occurs when

oxygenated blood is removed from (*e.g.*) heart tissue for a short period of time, and then returned. Injury develops quickly on return of the oxygenated blood flow causing (in the case of heart tissue), a reduction of heart rate and thus a reduction of blood flow. If the tissue is exposed to a solution of THII in the blood before ischaemia, this loss of function is inhibited. Although this study is in the preliminary phase, this property has great potential in the development of a medicinal agent.

vi) As yet, there is no evidence of any toxicity of the indenoindoles in animals.

4:1:1 *Antioxidation*

In chapter 1, it was mentioned that two *in vitro* systems were used to investigate the antioxidative properties of the dietary indoles. For the DHII and THII series only one was used; this demonstrates the ability of the indenoindoles to inhibit the lipid peroxidation initiated by iron(II) in the presence of ascorbate as described below.

Semi-purified asolectin (soybean phospholipid) is purified by precipitation from a chloroform solution using acetone: by repeating this procedure three times, it is possible to remove traces of tocopherols from the lipid. The purified lipid is stored in a chloroform solution and protected under an atmosphere of argon, at -20°C prior to use. Just before use, the solution is mixed with forty volume equivalents of acetone, the resulting precipitate collected by centrifugation, and dried under vacuum.

Typically, the purified lipid (12.5mg) is added to potassium phosphate buffer (pH 7.4, 6.25cm^3), and this suspension flushed with argon, sealed, and sonicated for 1 minute, or until the solution was translucent. The solution is then added to a reaction mixture containing ascorbic acid, iron(II)ammonium

sulphate, and varying amounts of the potential inhibitor. After a set time (usually 30 minutes), the reaction is stopped by the addition of BHT in DMSO. The amount of peroxidation achieved by the system can then be assayed[2] and the percentage inhibition (*vs* a control) evaluated.

A plot of inhibition *vs* concentration of inhibitor added, was made for all of the compounds assayed, from which it was possible to extrapolate the concentration of each compound required to bring about 50% inhibition of peroxidation; these are the values quoted in table 4:1.

From the results, we are able at least, in part, to deduce which aspects of the indenoindole structure enhance the activity. Ease of oxidation of the compounds does not appear to be the only contributing factor, although the non-aromatic secondary amines in the THII series are easier to oxidise than the heterocyclic amines of the DHII compounds, and are usually more active. However, the substitution of two methyl groups onto the methylene group at C-10 of DHII, or at 4b and 9b in THII should not affect greatly the oxidation potential of the compounds, yet there is an increase in activity.

Another factor might be the steric isolation imparted to these compounds, which protects the oxidised species formed from further attack. The position of the methylene bridging group in DHII and THII seems to be unimportant since the ~~geometric~~ STRUCTURAL isomers *iso*-DHII and *iso*-THII, respectively, exhibit identical activities. In the DHII series the activity of 10-methyl-DHII (71) is little changed from the parent, whereas 10,10-dimethyl-DHII (72) has one of the best activities measured.

The radical, or radical cation, formed from DHII is delocalised over the π-system of the indolic ring, and also over the benzenoid ring *via* the 4a-4b bond.

Table 4:1

Antioxidation activity of the indenoindoles	
compound	50%I(Fe)μM
6,8-Dimethyl-THII (136)	0.045
N-Methyl-THII (109)	0.06
10,10-Dimethyl-DHII (72)	0.06
4b,9b-Dimethyl-THII (119)	0.067
8-Methoxy-*N*-Methyl-THII (143)	0.067
8-Methoxy-THII (142)	0.067
Diphenylphenylenediamine	0.075
9b-Methyl-THII (118)	0.104
10,10-Dimethyl-THII (121)	0.12
iso-THII (102)	0.13
THII (100)	0.14
1,2,3,3a,4,8b-Hexahydrocyclopent[*b*]indole (99)	0.23
8-Methoxy-DHII (68)	0.65
6,8-Dimethyl-DHII (135)	0.89
BHT (6)	1.2
2,3-Dimethylindoline	1.2
1,2,3,4,4a,9b-Hexahydrocarbazole (97)	1.3
DHII (20)	1.5
iso-DHII (101)	1.5
10-Methyl-DHII (71)	2.0
8-Fluoro-DHII (67)	2.5
Indoline	3.8
1,2,3,4-Tetrahydrocyclopent[*b*]indole (98)	4.6

Table 4:1 continued

compound	50%I(Fe)μM
6–Chloro–DHII (69)	7
N–Methyl–DHII (66)	8.5
1,2,3,4–Tetrahydrocarbazole (28)	8.5
α–Tocopherol (103)	10
2,3–Dimethylindole	20
Indole–3–carbinol (1)	160
Indole	800
N–Acetyl–THII (112)	*ca.* 1800

At this time we are uncertain of the electron densities associated with such a delocalised species, but dehydrodimerisation can occur *via* C–9b (see section 3:3:3). This process can be sterically hindered by alkyl group substitution at C–10, and this causes the activity to rise accordingly. Therefore, mono–substitution has little effect on the activity, and di–substitution a much greater effect.

Nitrogen based radicals are stabilised by *N*–substitution. However for DHII, activity decreases on *N*–methylation. In chapter 1, we reported that in a study of 3–benzylindoles, both disubstitution of the bridging methylene group, and *N*–methylation reduces activity. Unfortunately 5,10,10–trimethyl–DHII was not synthesised as part of this work, but it seems likely that this compound would exhibit very little, if any, activity.

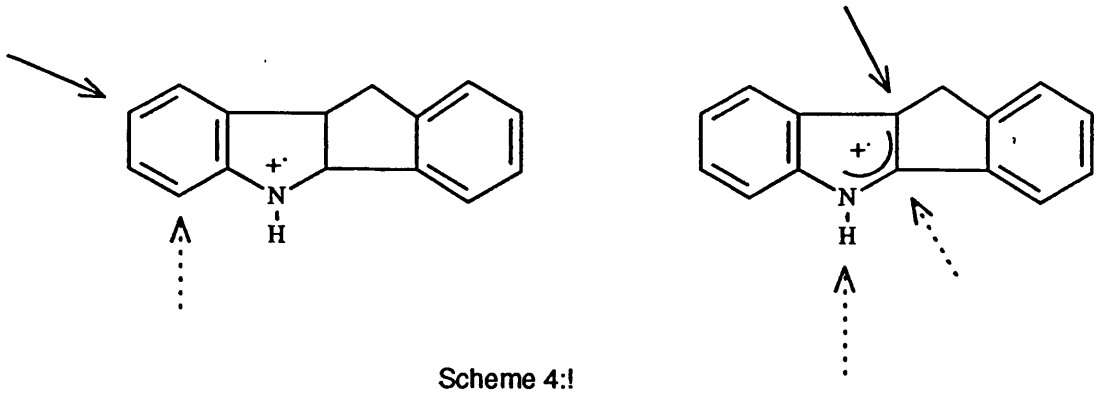
Substitution in ring A (the indole carbocycle) has a small effect on activity although, so far, the selection of functions is limited, and no compounds bearing an electron withdrawing (–M) group have been assayed. Thus at the present we do not know if this effect is resonance or sterically inspired.

For the radical of THII, the right hand benzene ring cannot be involved in the delocalisation of the unpaired electron, but interestingly *N*–methylation causes a 2 fold increase in activity. Further, although a methoxy group *para* to the nitrogen does enhance the activity of THII, *N*–methylation of this compound has no further effect.

As expected, 10,10–dimethyl–THII has the same biological activity as THII itself, for, of course, the C–10 position is isolated from the π–system of the presumed radical cation. However the 9b–methyl and the 4b,9b–dimethyl–THII are more active compounds and this may show that these methyl groups do provide some steric hindrance. Note that both 4b and 9b are benzylic positions, so that aryl radical cations formed from these molecules are unable to deprotonate as indicated in scheme 4:3, and so the extra stabilisation may be induced by the blocking of this reaction pathway (see section 4:2:1).

The best compound assayed in this series, is methylated at positions C–6 and C–8, but an even better compound should be the 4b,6,8,9b–tetramethyl analogue where not only are the sites for deprotonation blocked, but the oxidised species cannot be quenched by nucleophiles attacking the indoline system at positions *ortho* or *para* to the basic nitrogen atom. Unfortunately, we note that in the case of 4b,5,9b–trimethyl–THII, this compound is so easily oxidised, that it becomes coloured in air. Thus making an even more active compound may be counterproductive for a drug has to have a reasonable shelf life.

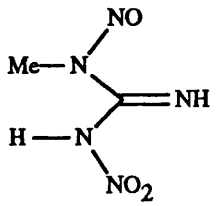
Although the antioxidation data has enabled us to deduce some information on the mode of action of the indenoindoles, it has revealed little about the fate of any intermediate radicals formed. However, we have some evidence that C–9b is a reactive position in the DHII series, whereas for the THII's, positions C–6, C–8, and C–4b seem to be important (scheme 4:1).



Scheme 4:I

4:1:2 *Stabilisation of Biological Membranes*

Table 4:2 relates the ability of the indenoindoles to protect cell membranes against lysis, and also to chemoprotect against the toxicity of 1–methyl–3–nitro–1–nitrosoguanidine (MNNG, 144), a potent mutagen and cancer suspect agent.



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To assay this former property of the indenoindoles, an osmotic fragility assay is used. For this, red blood cells containing heparinised blood, with or without a potential stabiliser, are infused with blood containing distilled water. When this is added, the cells swell under the osmotic pressure, and lyse. This lysis is reduced in the presence of a membrane stabiliser, and the efficacy of this stabilisation is quantified as a percentage protection of lysis per micromole of added compound. The indenoindoles all have values for this stabilisation of about 1.0–1.3 %/μM. These values show importance when they are compared with the values for the chemoprotective properties of the compounds against MNNG.

The latter assay involves the protection of isolated rat hepatocytes against

Table 4:2

RBC Fragility and MNNG protection data of indenoindoles		
compound	RBC fragility %/μM	MNNG protect μM/50% kill + 1Hr
N-Methyl-THII	1.14	1.6
10,10-Dimethyl-DHII	0.995	
Diphenylphenylenediamine	1.202	2.05
9b-Methyl-THII	0.301	
10,10-Dimethyl-THII	0.843	
iso-THII	1.13	2
THII	1.24	2.2
1,2,3,3a,4,8b-Hexahydrocyclopent[*b*]indole	0.31	5.0
8-Methoxy-DHII	0.50	
BHT	1.80	10
1,2,3,4,4a,9b-Hexahydrocarbazole	0.288	
DHII	0.99	3.1
iso-DHII	1.18	0.98
8-Fluoro-DHII	0.64	
Indoline	0.54	
1,2,3,4-Tetrahydrocyclopent[*b*]indole	0.42	15.5
6-Chloro-DHII	0.445	
N-Methyl-DHII	1.48	6
1,2,3,4-Tetrahydrocarbazole	0.56	
α-Tocopherol	0.12	161
Indole-3-carbinol	0.10	226
N-Acetyl-THII	0.572	

N.B. The compounds listed are in the same order as for table 4:1.

the toxic agent. The cells – in the presence of 0.5mM of MNNG – have a half life of about 45 minutes; figure 4:1 shows the extension of this lifetime in the presence of varying amounts of THII. The efficacy of this chemoprotection, is measured as the amount of compound required to extend the halflife of the hepatocytes by 60 minutes.

When these two properties are compared (figure 4:2), it is possible to show a link between the RBC fragility assay, and the chemoprotection against MNNG. This reveals that there is an optimum value for the stabilising effect of about 1.14%/μM. Values above or below this, tend to render a compound as a less efficient chemoprotective agent. The indenoindole compounds all have values around this optimal point.

RBC fragility is probably linked to the lipophilicity of the compounds, fig. 4:2 suggests that the molecules already tested are sufficiently lipophilic, and any change in this property would adversely affect the chemoprotection mentioned above. Certainly Professor Shertzer has suggested that there is no need to improve the lipophilicity of our compounds by the addition of long chain alkyl groups, as described in chapter 3.

4:1:3 *Toxicity of the indenoindoles*

If the indeno[1,2-*b*]indole compounds are to have any utility as *in vivo* antioxidants, it follows that they must exhibit very little toxicity. In order to evaluate their intrinsic toxicity, some initial tests have been undertaken, primarily on DHII.

In one such test, mice were treated by gavage with one of the three dietary indoles – indole-3-carbinol (I-3-C), or with DHII, and then had their behaviour monitored over a 24 hour period. In the case of I-3-C, effects of the admin-

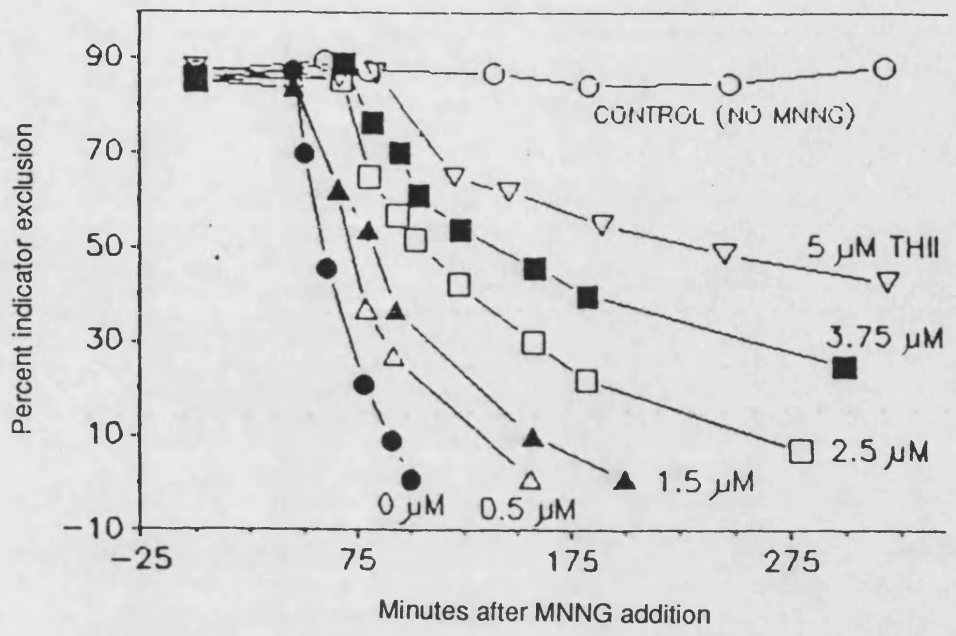


Figure 4:1

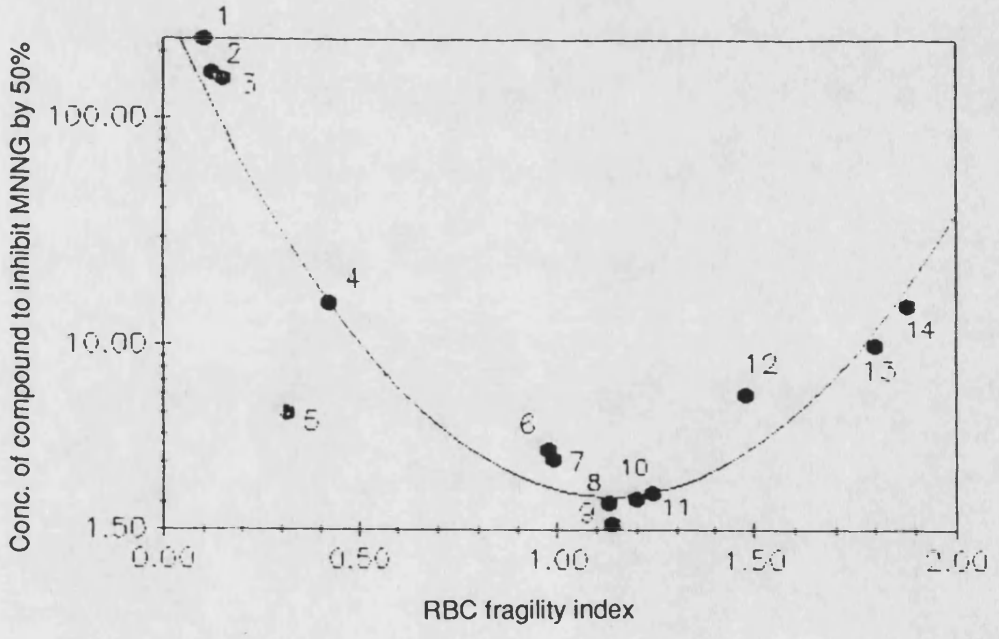


Figure 4:2

1. I-3-C
2. Vitamin E
3. Indole-3-ethanol
4. HHCPI
5. THCPI
6. iso-DHII
7. DHII
8. iso-THII
9. N-Me-THII
10. DPPD
11. THII
12. N-Me-DHII
13. BHT
14. Promethazine

istered compound were noticed at a dose of 0.1g per kg bodyweight, and the compound induced a comatose state at a dose of 0.5g kg^{-1}. The mice treated with DHII however, showed very little, if any, effect in their behavioural activity with doses as high as 4.0g kg^{-1} (figure 4:3).

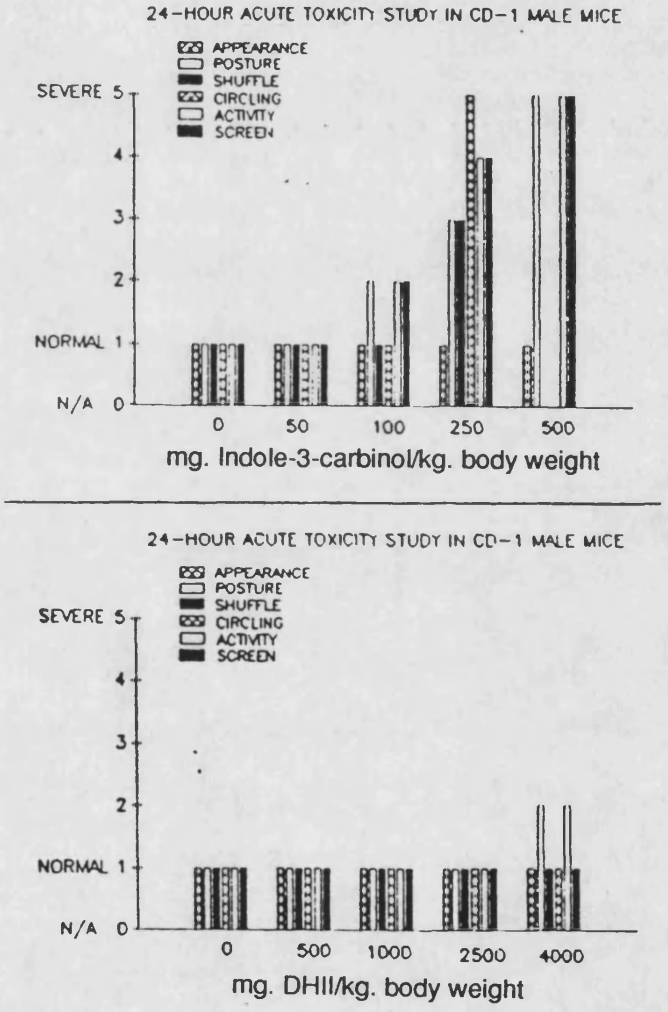


Figure 4:3

Similar tests are being undertaken on mice, with THII. Preliminary results indicate that the urine of the test animals is coloured after treatment with these

compounds. Considering our evidence of radical cation/radical formation within the THII series (section 4:2:4), this could indicate that the compounds are acting as *in vivo* antioxidants, to form stable radical species, which are discharged harmlessly by the normal metabolic processes.

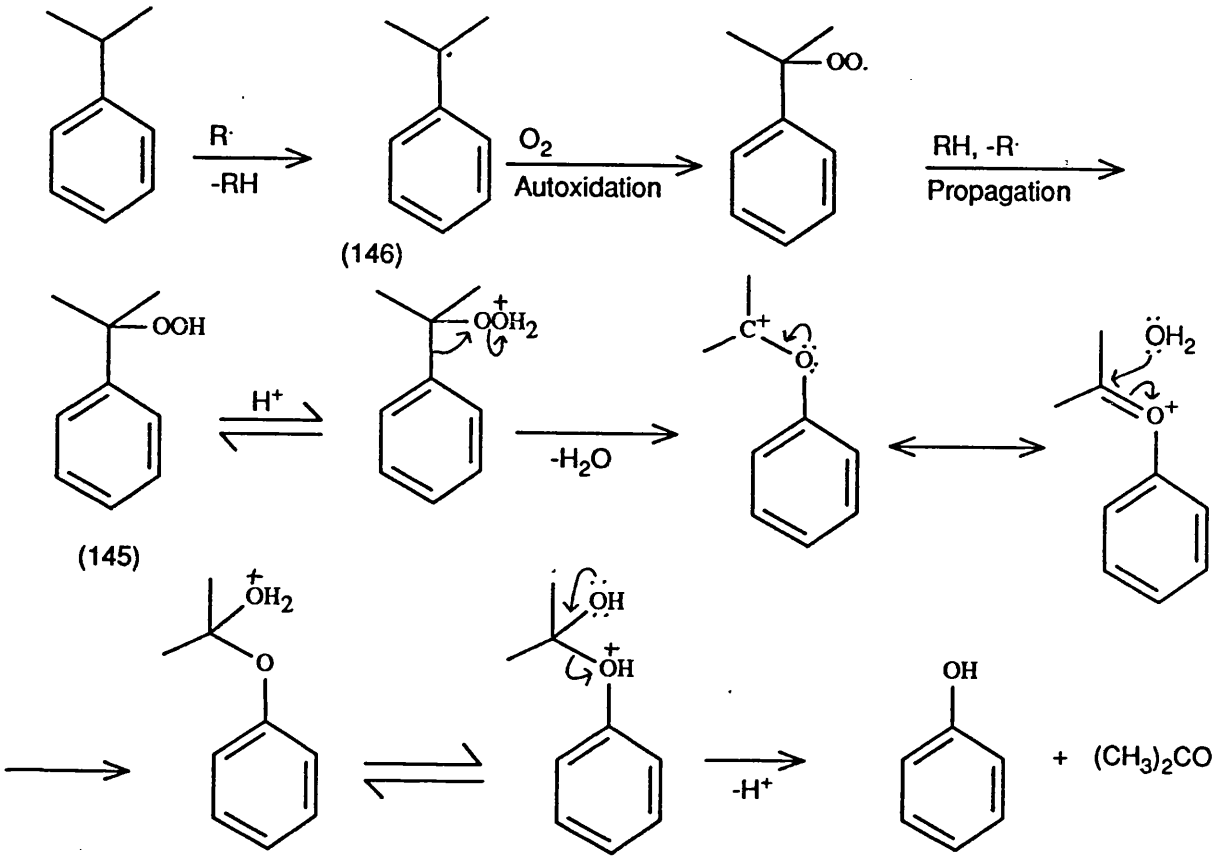
4:2 Chemistry of indenoindoles

4:2:1 *Inhibition of the Cumene Process*

Having determined that the indenoindoles inhibit *in vivo* oxidation, a non-biological model was sought, in order to demonstrate whether the THII family of compounds could find some use as antioxidants in industrial processes. The potential market for this is very large; it includes protection of rubber products, petroleum fuels, and cooking oils (where, of course, toxicology becomes a major factor).

Autoxidation has many useful applications[3] as, for example, in the synthesis of phenol and acetone from cumene (scheme 4:2). Here the initially formed hydroperoxide (145), undergoes an acid promoted rearrangement to give phenol and acetone in very good yield. This process is one of the main methods for the industrial production of phenol.

The first step in this process is initiated by free radicals generated, for example, by the thermolysis of AIBN. Since the cumenyl radical (146) is both benzylic and tertiary, its formation is easily achieved, and may be followed conveniently by ^{1}H nmr spectroscopy. Thus, whereas the methyl protons (2') of cumene resonate as a doublet at 1.25ppm (^3J =7Hz), coupled to a septet at 2.9ppm due to the methine hydrogen atom (1'). The spectrum of cumene hydroperoxide, on the other hand, lacks this signal, and now the methyl protons resonate as a singlet at 1.7ppm (I). This is clearly noted in the spectrum of



Scheme 4:2

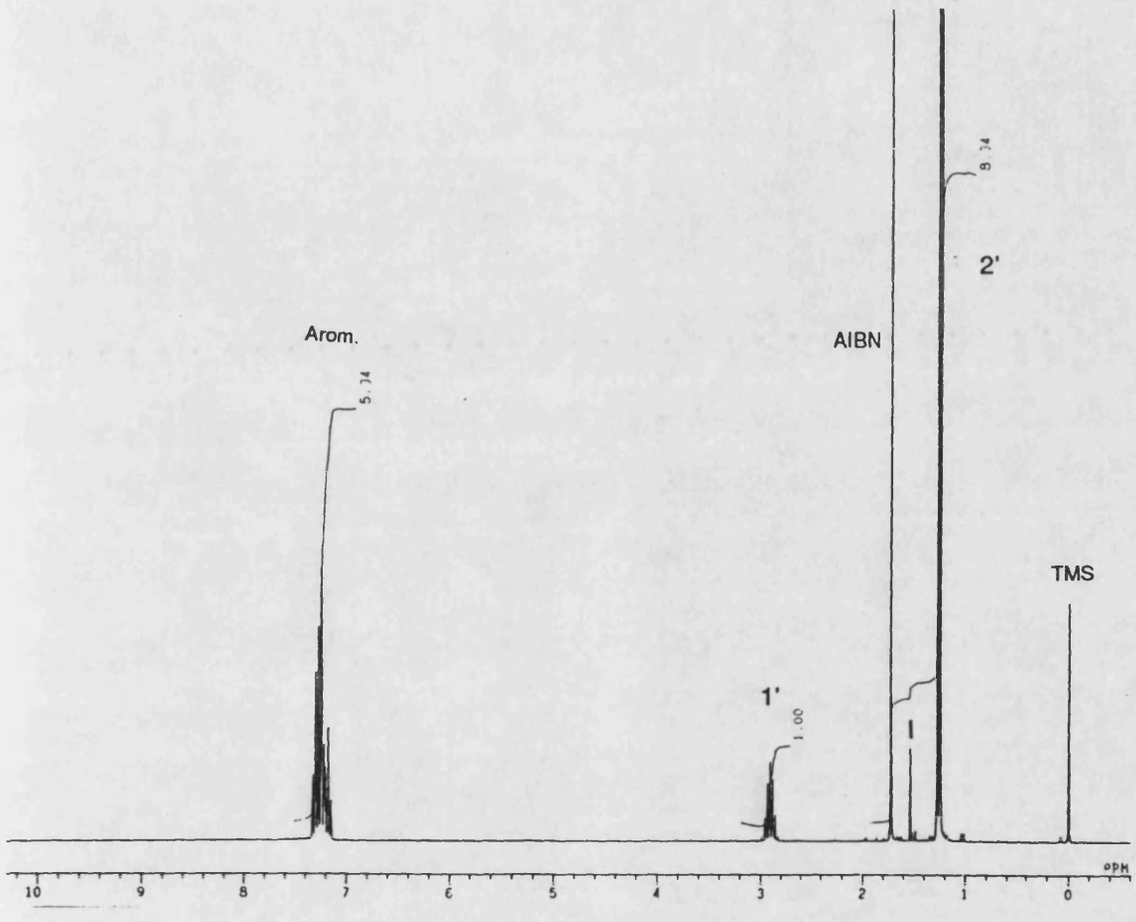
cumene after exposure to AIBN, and the intensity of this signal is observed to increase if the length of the experiment is extended (as fig 4:4 demonstrates after 20 hours oxidation). An antioxidant should prevent the formation of the cumenyl radical, and we considered that the inhibition of the cumene/AIBN system might be used as a test for the behaviour of our compounds.

In the first experiment, one molar equivalent of THII (100) was added to a solution of cumene in deuterochloroform containing 10 molar percent of AIBN. The sample was sealed under an atmosphere of oxygen, and shaken vigorously. The spectrum of the reaction mixture was recorded after 20 hours, and we noted that it consisted of a composite of the individual spectra of cumene, AIBN, and THII (fig 4:5). The proton resonances of THII are numbered in accordance with our assignments (see experimental), and those of AIBN and cumene are assigned

as in fig 4:4. No cumene hydroperoxide was detected. One point worthy of note, however, was that the N–H resonance of THII at *ca.* 4.0ppm, was broadened to the point where it merged with the baseline. It is known that the presence of a radical in a ^{1}H nmr sample decreases the relaxation time of resonating nuclei, and if autoxidation has been inhibited, it follows that a radical or radical cation is generated from the inhibitor. Thus it is possible that the N–H resonance is experiencing an effect due to chemically induced dynamic nuclear polarisation (CIDNP)[4] from such a paramagnetic species which is present at low concentration.

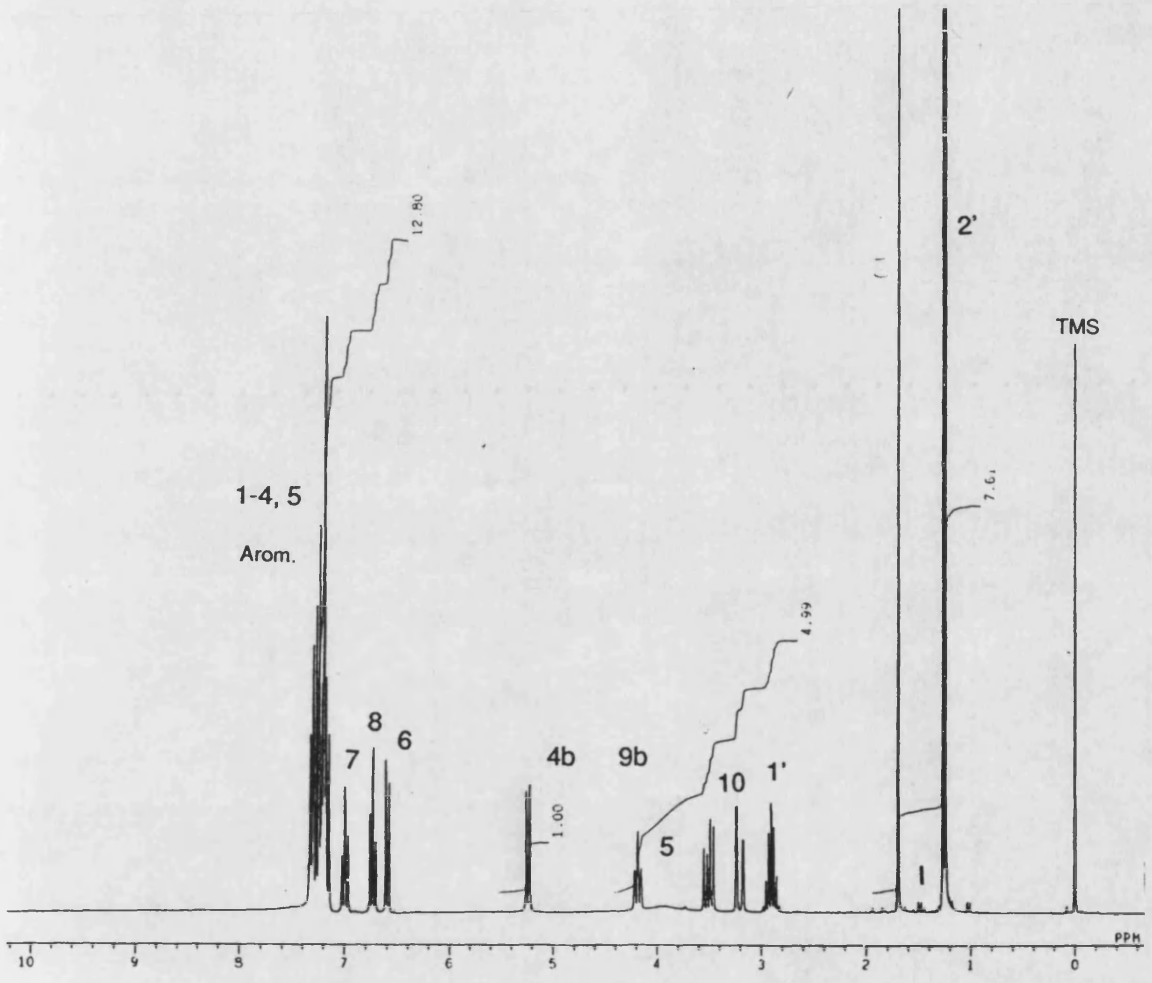
This experiment was continued for a week, and the ^{1}H nmr spectrum re–recorded. Even after this period, the presence of cumene hydroperoxide was not detected. The spectrum is shown in fig 4:6, and it is interesting that the N–H resonance is now much less broad. Closer examination of the N–H signal shows an unexpected extra resonance at 3.7ppm due to a low concentration of DHII (20) (this singlet is due to the resonance of the C–10 methylene hydrogen atoms, all the other signals are hidden in the aromatic envelope). This finding was confirmed by analysis of the mixture by g.l.c., and a repetition of the experiment with *N*–Me–THII (109) in place of THII from which very similar results were obtained (fig 4:7). The spectrum in this experiment is more broadened than with THII, indicating a greater concentration of unpaired electrons. Again no sign of any oxidised species are indicated (this spectrum was taken after 1 week, similar to fig 4:6), and the two extra peaks (X) at 4.0 and 3.7ppm are due to the N–Me and the C–10 methylene protons of *N*–Me–DHII respectively.

These results allow us to conclude that the initial step is a competition between cumene and THII (or *N*–Me–THII) for the initiating radicals (In·) from AIBN. The THII compounds are successful, and react to generate a relatively stable radical (relative to the cumyl radical), perhaps by hydrogen abstraction at



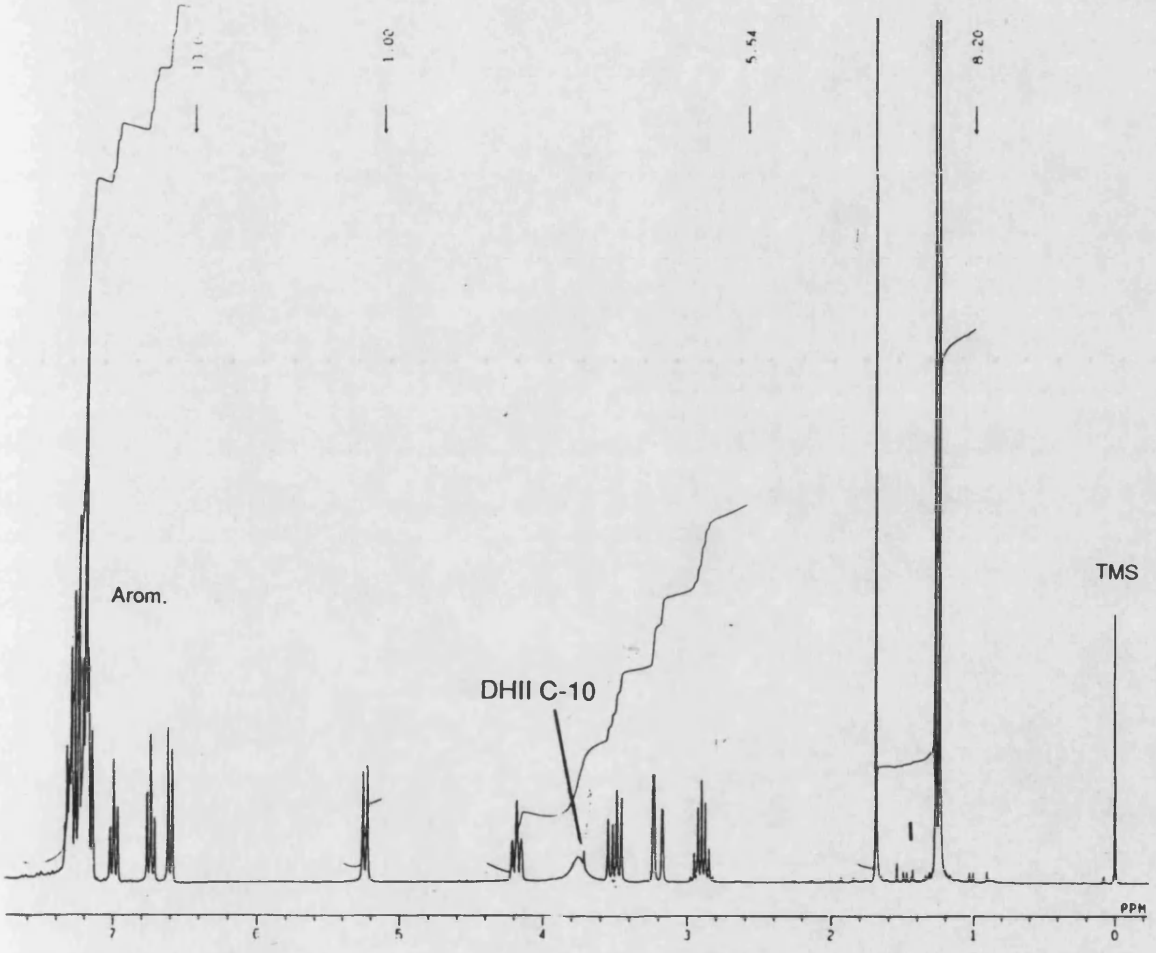
Autoxidation of cumene initiated by AIBN

Figure 4:4



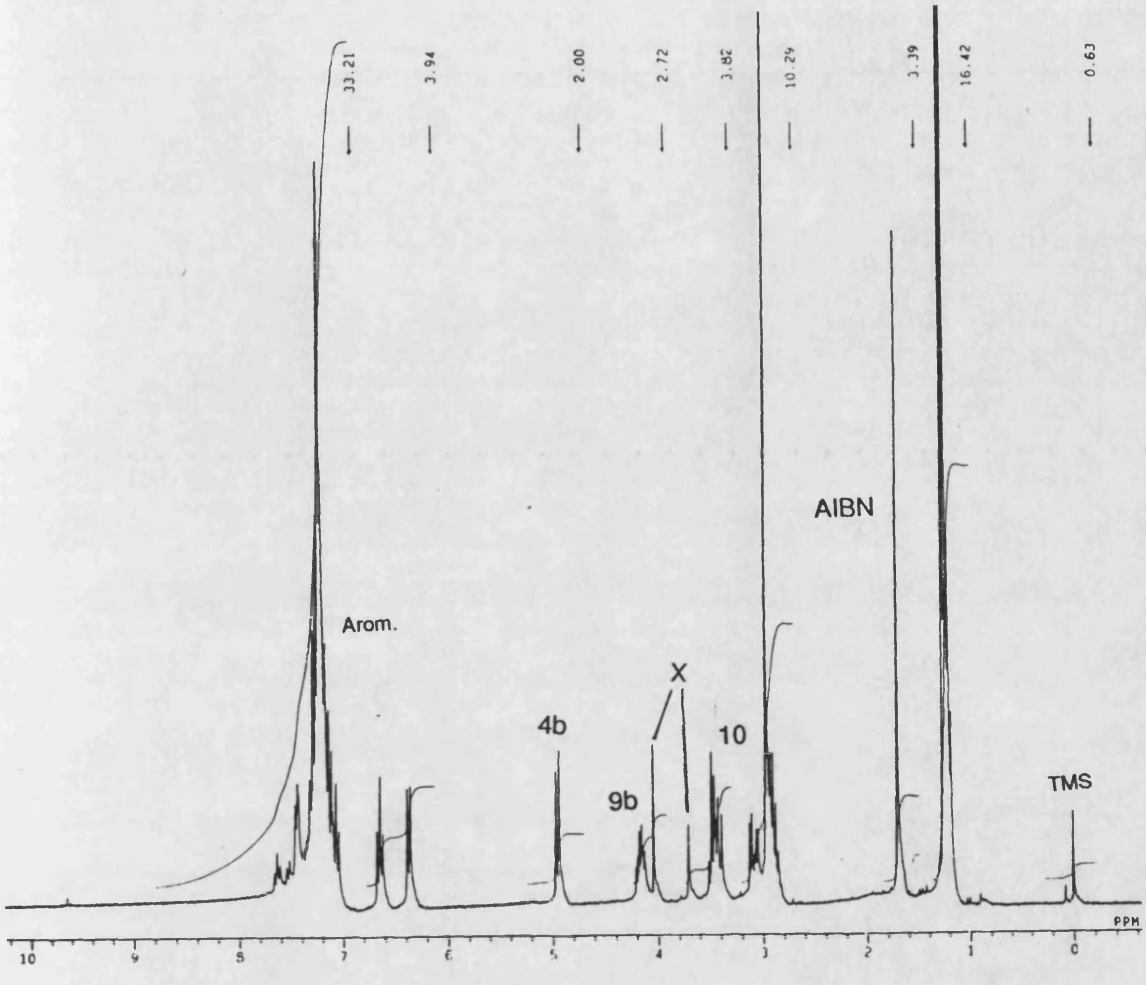
Autoxidation inhibited by THII - 20 hours

Figure 4:5



Autoxidation inhibited by THII - 1 week

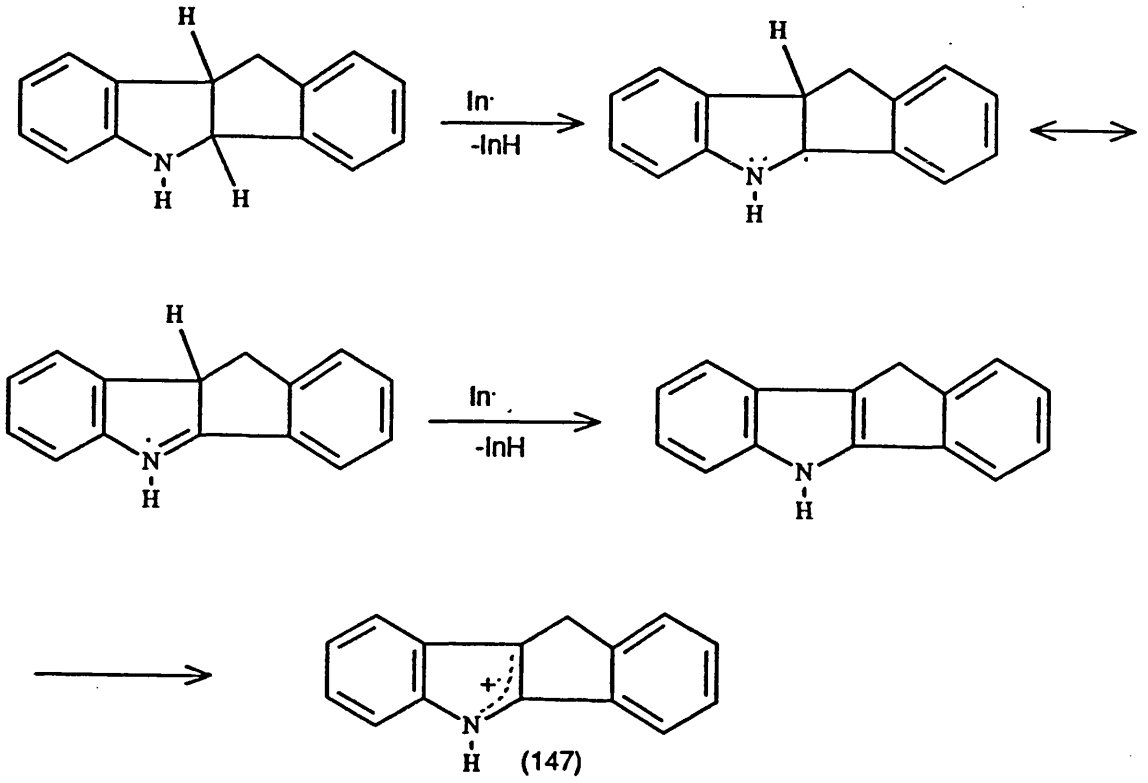
Figure 4:6



Autioxidation inhibited by N-Me-THII - 1 week

Figure 4:7

C–4a (scheme 4:3). These radicals then decay by further hydrogen loss, to give DHII, or its analogue *N*–Me–DHII depending on the initial starting material.



Scheme 4:3

Since the DHII series of compounds are also antioxidants, these may react further to afford radical cations of the type (147), which may then decompose perhaps by dimerisation *etc.*

During the course of the cumene reactions, we observed that the solutions of the reactants became highly coloured; that of THII becoming dark red, and that of *N*–Me–THII becoming dark blue. Such effects also suggest the production and presence of free radicals, and led us to investigate the esr spectra of the solutions. Low concentrations of radicals are in fact present, and the results of these investigations are given in section 4:2:3.

At an anode, a molecule transfers a single electron from its HOMO to the electrode, thus forming a radical cation. This species may immediately react, or undergo further electron loss as the potential is raised. Thus there is a important distinction between radical formation by electrochemistry, and by formation with initiators where the radical is formed by hydrogen atom abstraction as, for example, in the cumene experiments.

As we described in section 4:1:1, the initial *in vitro* test by which Professor Shertzer ranked our antioxidants, is the Fe/ascorbate assay. It is believed that the oxidation is promoted by one electron transfer to ferric ion, representing the type of oxidation that may be caused by cytochrome P450 systems *in vivo*.

There is much experience within this group with the electro–oxidation of hetero–aryl systems, and so it occurred to us to attempt to correlate ionisation potential (from anodic oxidation) with the results of the Fe/ascorbate assay. For this purpose, we used the technique of cyclic voltammetry. Here the substrate contained in a supporting electrolyte (acetonitrile/sodium perchlorate is commonly used) is electrolysed in a small cell containing a platinum bead working electrode, a reference standard calomel electrode (SCE), and a large surface counter electrode. The potential of the bead is increased, and the potential difference between it and the SCE is monitored, as well as the cell current. It is possible to sweep in both anodic and cathodic directions, and the name derives from the fact that it is common to sweep first anodically from 0 to X volts, and then to reverse the polarity, and sweep cathodically from 0 to -X volts. Since the only electroactive species in the cell are associated with the substrate, then as this, or its products, become oxidised or reduced, peaks due to the electron transfer processes become evident in the voltage/current trace.

Clearly, not only does this technique provide data about ionisation potentials, it also gives much information about events which take place after ionisa-

tion. For example, if the initially generated cation radical formed in the anodic sweep is stable, it remains to be reduced in the reversed cathodic sweep. A characteristic redox couple is then apparent, with approximately equal sized peaks at equal but opposite potentials. Should the initial radical cation be unstable, information may also be gleaned about its fate, and the nature of the product it forms.

Initially we sought only to obtain first ionisation potentials, and table 4:3 summarises a series of results for some of the 3-benzylindoles, along with the corresponding Fe/ascorbate data. The first point to make, is that with one exception, all the compounds show essentially the same first oxidation potential despite the fact that the benzylic substituents range from electron donating compounds (7 and 11), to an electron withdrawing compound (9) (this technique may be assumed to have an error of at least 0.05v).

Table 4:3

CV data on 3-benzyl-indoles		
Compound	Ox. pot. (v)	50% I (Fe) μM
3-benzylindole (6)	1.06	36
3-(4-hydroxybenzyl)indole (11)	1.01	12
3-(4-methoxybenzyl)indole (7)	1.07	24
3-(4-dimethylaminobenzyl)indole (12)	0.65	1.5
3-(3-pyridinylmethyl)indole (9)	1.00	500
1-methyl-3-(2-methyl-2-phenyl)indole	1.02	>2500

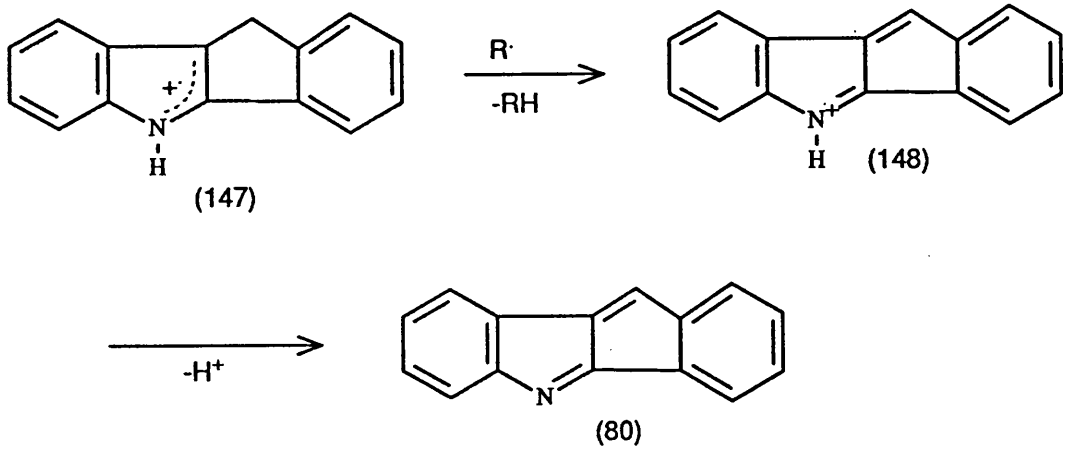
It is clear therefore that the site of electron loss is the indole heteroaryl system (for 3-(4-*N*,*N*-dimethylbenzyl)indole, the anomalously low ionisation figure

is due to ionisation from the lone pair on the dimethylamino substituent).

It is most interesting that the *N*,*N*–dimethylaminobenzyl compound has the highest activity in the Fe/ascorbate assay, and that isolation of the indolyl 'or phenyl moieties causes complete loss of activity. These results do indicate that ionisation potential is not the sole factor deducing the activity of these compounds, and other factors such as hydrophobicity may be equally important.

Nonetheless, we felt that the correlation might be useful in ranking the compounds in the DHII/THII series, where we had rather more examples.

For DHII, one might anticipate that the extended conjugation imparted by the indol–2–yl–benzene bond of the fused system, would raise the energy of the HOMO, and hence lower its ionisation potential. This is indeed so, and DHII is oxidised at +0.78 volts. It also shows the same biological activity as 3–(4–*N*,*N*–dimethylaminobenzyl)indole, the "best" of the simple 3–benzylindoles. Could it really be that low ionisation potential is directly equatable with high activity in the Fe/ascorbate test? In all the examples, it is possible to visualise the initially formed radical cations (147) losing a hydrogen radical (H·) from the benzylic position, to give cations (148), which may *N*–deprotonate to give the fully conjugated compounds (80), (scheme 4:4).



(147) (148) (80)

Scheme 4:4

However, as we discussed in chapter 3, such species are antiaromatic and intrinsically of high energy. This factor favours other fates for the cationic or neutral radicals, such as dimerisation.

If the radical cations have some stability, then their reduction on the reverse sweep during cv should be apparent. Unfortunately, the anodic/cathodic peaks are broad, and it is difficult to decide the outcome of the experiments. In the case of DHII for example (fig 4:8, trace 1), the cv trace illustrates some symmetry relating the shape of the oxidative sweep (upper) to that of the reductive sweep (lower). However, this reversibility could be due to the reduction/oxidation of product (or products), as well as that of the substrate, since the curves do not give sharp peaks, and instead cover a range of potentials.

It was the apparent relationship between oxidisability and antioxidant effect as measured by the Fe/ascorbate test, which caused us to examine the THII series of compounds, since now these compounds are related to *N*-methylanilines, and thus should oxidise at similar potentials to 3-(*N*,*N*-dimethylaminobenzyl)indole.

Some data for the oxidation/activity of derivatives of THII, as well as for other DHII compounds are listed in table 4:4, along with the values of two reference compounds, tetrahydrocarbazole (THC, 28), and hexahydrocarbazole (HHC, 137).

As expected, when THII is *N*-acylated, its oxidation potential is raised, and activity is lost. Conversely, when there is an electron donating group in ring A *para* to the nitrogen atom of the heterocycle, the oxidation potential is lowered, and activity is raised.

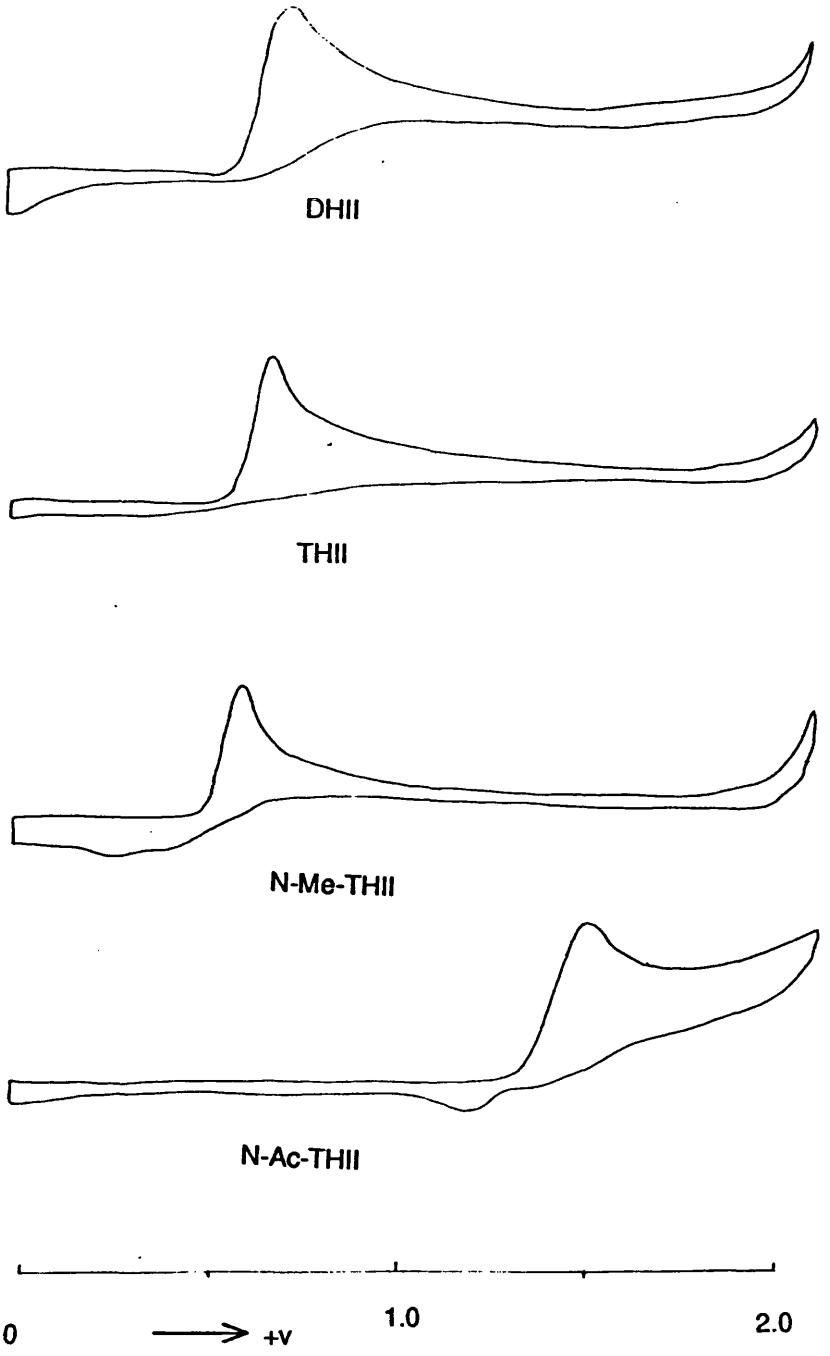
The most active compounds are not however the easiest to ionise. Pride of place goes to 10,10-dimethyl-DHII, and *N*-methyl-THII, which both inhibit the Fe/ascorbate system at the low concentration of 0.06μM. Fig 4:8, trace 3 is that

Table 4:4

CV data on indeno[1,2-*b*]indoles		
Compound	Ox. pot. (v)	50% I (Fe) μM
DHII (20)	0.78	1.5
THII (100)	0.67	0.14
6,8-DM-DHII (135)	0.65	
6,8-DM-THII (136)	0.57	
10,10-DM-DHII (72)	0.77	0.06
10,10-DM-THII (121)	0.68	0.12
N-Me-THII (109)	0.58	0.06
N-Ac-THII (112)	1.475	1800
8-MeO-DHII (68)	0.75	0.65
8-MeO-THII (142)	0.46	
8-MeO-5-Me-THII (143)	0.395	
4b,9b-DM-THII (119)	0.71	
4b,5,9b-TM-THII (120)	0.62	
THC (28)	0.77	8.5
HHC (98)	0.71	1.3

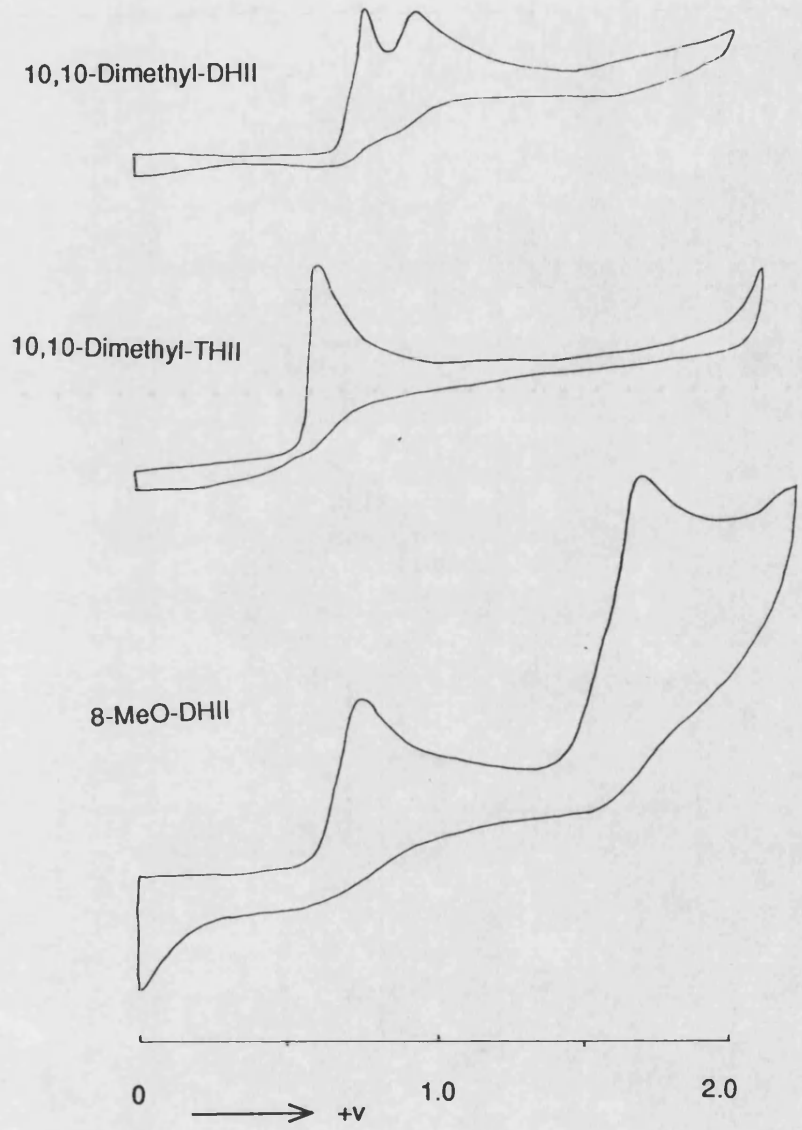
for the latter compound, whereas 10,10-dimethyl-DHII and its THII analogue are shown in fig 4:9; the third trace in fig 4:9 is that for 8-methoxy-DHII.

An interesting feature of the first trace, is the formation of a product peak at 0.95 volts. This seems to be relatively long lived, for there is an inflexion in the reductive trace to indicate its reduction at -0.88 volts. The nature of this "product" remains unsettled, it cannot be simply the radical formed by *N*-



CV traces of the indenoindoles

Figure 4:8



CV traces of substituted indenoindoles

Figure 4:9

deprotonation of the initial radical cation, for this species would surely not be more difficult to ionise than the initial substrate itself.

A second anodic peak also appears in the voltammogram for 8-methoxy-DHII, but this is at such a high potential that it is probably due to a second ionisation of the substrate, probably at the methoxy substituent.

The discrepancy between the activity between 10,10-dimethyl-DHII, and its THII analogue, indicates that there are two separate mechanisms for the fate of these two molecules. The indole was much more active than its parent, whilst the compound was expected to be slightly less active. The cv traces indicate that the first ionisation potential of the two indoles are the same (within experimental error), and so ease of oxidation is not the major contributing factor. Commercial antioxidants such as BHT (6), and BHA (7), are considered to act by forming stable free radicals which are "protected" by resonance, and by steric hindrance to the approach of potential substrates; therefore causing chain termination. Thus it could be that by having the two simple alkyl groups on the methylene bridge of DHII, we are shielding the radical substrate from further reaction. This would therefore suggest that the radical once formed in the indole series, is conjugated with the pyrrole double bond, and that any further reaction takes place through the positions on this ring (scheme 4:1). Any attacking substrate would have to approach the substrate from above, or below, the ring system, and would be prevented from doing this by the presence of the alkyl groups. Thus we have protected the molecule from attack at the 9b position, and by doing so formed a more stable radical species, which is thus a better antioxidant.

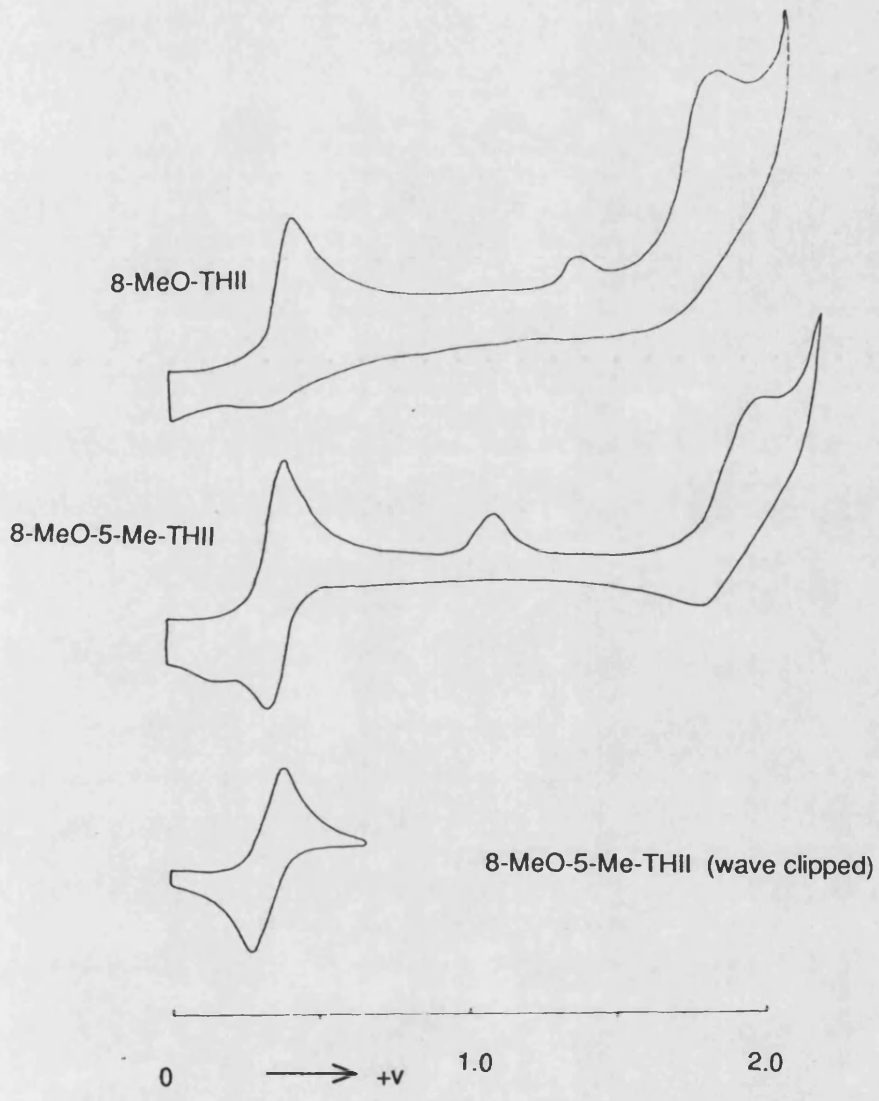
If a more stable radical is formed as a result of the electro-oxidation, as we explained earlier, we would expect to see a reduction peak of this species during the reversed sweep. This we do not see thus suggesting that there are more sites through which the molecule may react. The obvious candidate for this further

reaction is the nitrogen atom which is distant enough from the C-10 position not to experience the shielding. We could maybe prevent any reaction on the nitrogen by substituting a simple alkyl group for the hydrogen. However, the results obtained in the previous work on the 3-benzylindoles showed that 1-methyl-3-(2-methyl-2-phenylethyl)indole had virtually no activity in the Fe/ascorbate assay, and it was thought that the equivalent fused compound would also exhibit no activity.

Conversely, the data for 10,10-dimethyl-THII corresponds very closely with that for THII itself, thus suggesting that the two alkyl substituents have no effect on the activity of the molecule. The trace for the substituted compound also is very similar for that of THII (fig 4:9, trace 2). This suggests that the radical formed on the nitrogen is associated with the six-membered ring (which is to be expected when the aromaticity of the pyrrole is removed. A logical step to "protect" the radical formed in the best of our antioxidants in the THII series, is to increase the substitution of the compounds both to improve the mesomerism and the steric protection of the compounds.

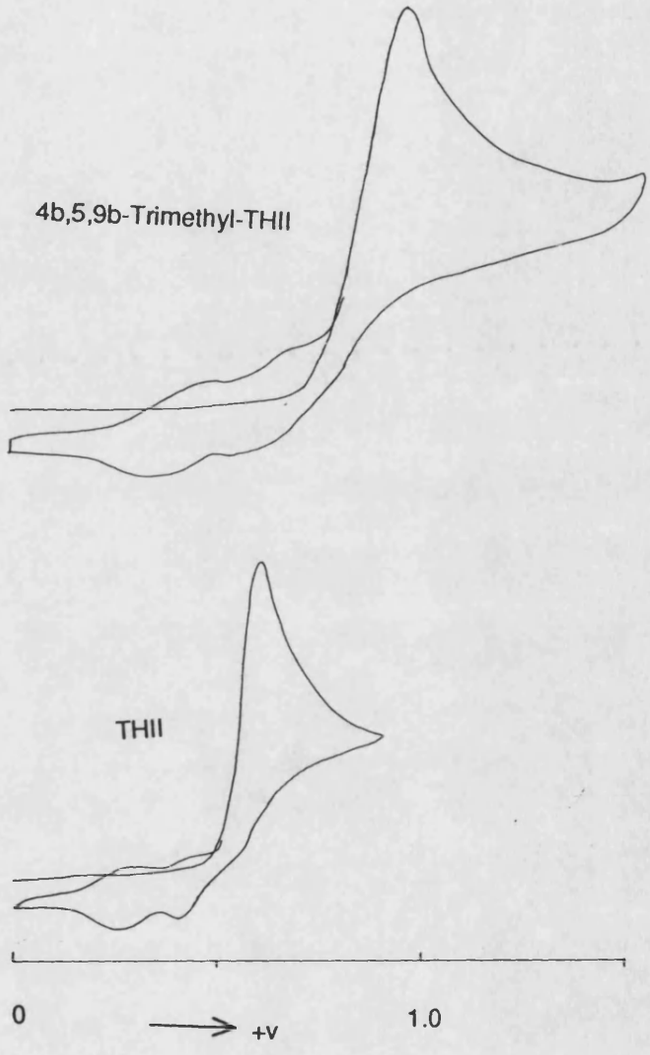
A good example of how this is achieved is shown by the cv traces of 8-methoxy-THII, and 8-methoxy-5-methyl-THII (fig 4:10). The first compound gives a trace very similar in many aspects to the DHII analogue (fig 4:9, trace 3), although there is evidence that this radical cation survives longer than some other examples to provide a partial redox couple. On the other hand, once the nitrogen atom is *N*-methylated, an almost perfect redox couple is produced, marred only by a small reduction peak at low potential. This feature is associated with products of over oxidation , for if the voltammogram is "wave clipped" (a technique by which the voltages are swept over a small range), a perfect couple is achieved (fig 4:10, trace 3).

A feature common to many of the THII derivatives, is the appearance in



CV traces of alkoxylated THII

Figure 4:10



CV traces of indenoindoles in DCM/TBATFB

Figure 4:11

their cv traces of a redox couple at a lower potential than that of the first ionisation. Obviously this is associated with a product, for they are not present on the first sweep. Thus far, the nature of these species has not been established (for example see fig 11 which demonstrates the cv traces of 4b,5,9b–THII (120), and THII taken over two sweeps).

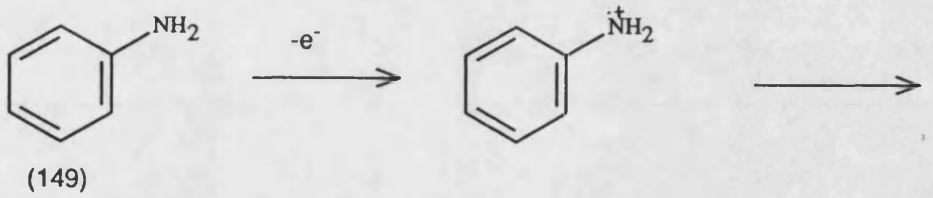
However aniline (149) is known to be oxidised at *ca.* 1.0v, but a redox couple is not formed.[5] Instead, two new cathodic peaks at *ca.* 0.7 and 0.4v appear on the second sweep, which are individually coupled to two new anodic peaks.

Anodic oxidation of aniline gives benzidene (150), and *p*–aminodiphenylamine (151) (scheme 4:5). These two products have oxidation potentials lower than that of aniline, and it is assumed that it is the oxidation and reduction of these two products which give rise to the two new peaks in the cv trace. By analogy, we might anticipate that similar dehydrodimers [*e.g.* (152) and (153)] form from coupling of the THII cation radicals. These are more highly conjugated and thus should have lower ionisation potentials than the parent "monomers", and these dehydrodimer "products" may be responsible for the low potential couples we observe in the cv traces.

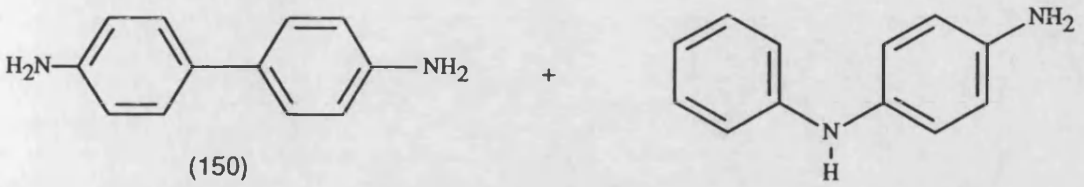
We have conducted preparative electrolyses with DHII, but even in the presence of nucleophiles, no tangible products were formed. With THII, a green material is formed. This seemingly impure substance gives an intense esr signal, even after some weeks storage.

4:2:3 *Radicals from the Indeno[1,2-b]indoles*

All through this work, we have suggested that that stability of indenoindole radical cations or radicals, has a direct relationship to bioactivity. Following on from this argument, it is expected that the most active compounds should have



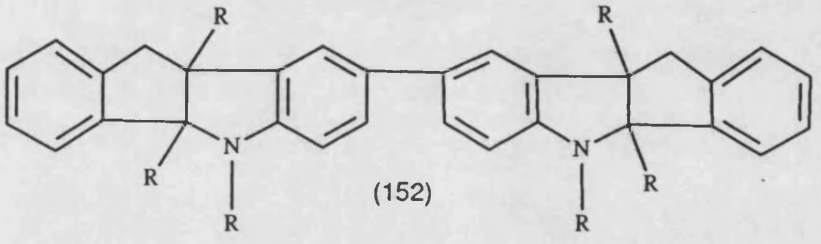
(149)



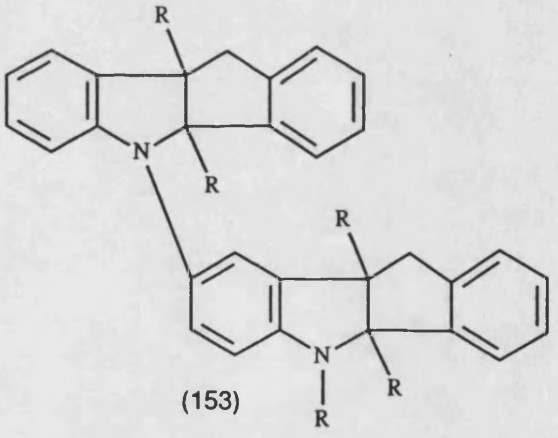
(150)

Scheme 4:5

(151)



(152)



(153)

the most persistent radicals. Such species should be detectable by esr spectrometry.

The first sample to be investigated was from the cumene oxidation experiments described in section 4:2:1 containing THII. The esr spectrum of the blue coloured solution of this species, exhibits a weak 1:1:1 triplet, which suggests that the unpaired electron is centered on the nitrogen atom. Unfortunately, further

hyperfine coupling (if present), is masked because of the low intensity of the signal. Since we know from the cv experiment that the radical cation of THII has a relatively short half life, it is clear that this persistent species must be due to a product, possibly a dehydrodimer bonded through nitrogen. For we were unable to detect a similar signal of a persistent radical in the equivalent experiment containing *N*–Me–THII. Interestingly, however, the ^{1}H nmr spectrum of this compound shows considerable line broadening.

In one last attempt to identify an oxidation product from THII, a preparative electrolysis was carried out. Here the platinum anode potential was controlled at 0.7v, and the supporting electrolyte was acetonitrile containing 5% sodium perchlorate. During the electrolysis, the solution turned very dark green in colour, and, on aqueous work–up, a green solid was deposited. Attempts to purify this material by chromatography were thwarted due to the materials insolubility in most organic solvents. Similarly, its ^{1}H nmr spectrum in DMSO–d_6 could not be analysed since none of the signals were resolved. The solid exhibited a broad (*ca* 30G) singlet in the esr spectrometer, which we were unable to resolve any further.

4:3 Future Work

We intend to synthesise several more analogues of THII in order to optimise the antioxidant properties of these compounds. In this work, we hope to unravel the fate of the oxidised compounds, and here preparative electrolysis in the presence of nucleophiles may be useful. This technique has been used previously to "capture" the positively charged products resulting from, say, the dimerisation of radical cations. Much else in this programme will depend on the results of the pharmacological tests which are about to begin, and it is clear that an immediate requirement will be the optical resolution of the THII compounds.

References to Chapter 4

1. R. O. Recknagel, E. A. Glende Jr., and A. M. Hruszkewycz, *Free Radicals Biol.*, 1977, **3**, 97.

2. H. G. Shertzer, M. L. Berger, and M. W. Tabor, *Biochem. Pharmacol.*, 1988, **37**, 333.

3. W. G. Lloyd, *Autoxidation,* in *Methods in Free Radical Chemistry*, ed. E. S. Huyser, vol. 4, p. 1, Marcel Dekker, New York (1973).

4. H. R. Ward, *Chemically Induced Dynamic Nuclear Polarisation,* in *Free Radicals*, ed. J. K. Kochi, vol. 1, p. 239, John Wiley, New York (1973).

5. J. Bacon and R. N. Adams, *J. Am. Chem. Soc.*, 1968, **90**, 6598.

5 Experimental to chapters 3 and 4

5:1 *General*

Solvents and Reagents

"Petrol (60–80°C)" refers to that fraction of petroleum ether boiling in the range 60–80°C; it was distilled prior to use. THF was distilled under an atmosphere of nitrogen, after drying with sodium in the presence of benzophenone, and diethyl ether was dried over sodium–lead alloy and then over sodium wire. Dichloromethane, benzene, and acetonitrile were distilled from calcium hydride, and triethylamine was distilled from calcium hydride and stored over 3Å molecular sieves. Other solvents and reagents were either purified by following the procedures outlined in *Purification of Laboratory Chemicals*,[1] or used in the form obtained from the chemical suppliers.

Chromatography

Medium pressure (Flash) column chromatography was used in general for the purification of reaction mixtures. "Silica gel" refers to Amicon Matrex 84072 silica gel (230–400 mesh), or Merck 9385 silica gel.

T.l.c. was used extensively for following the course of reactions, for the analysis of fractions from chromatographic columns and for assessing the purity of compounds. It was performed on aluminium plates coated with Kieselgel 60 F_{254} silica gel, and compounds were visualised in the first instance by illumination with short wavelength (254 nm) u.v. light. Thereafter visualisation was achieved using one of the following:

1. aqueous potassium permanganate solution.

Spray/dip solution: Potassium permanganate (3g), and potassium carbonate

(29g) were dissolved in 5% aqueous sodium hydroxide ($5cm^3$) and water ($300cm^3$).

2. 2,4–dinitrophenylhydrazine (DNP) for aldehydes and ketones.
Spray/dip solution: 2,4–DNP (12g) in concentrated H_2SO_4 ($60cm^3$) was added to water ($80cm^3$), and 95% ethanol ($200cm^3$).

3. iron (III) chloride in perchloric acid for indoles.
Spray/dip solution: 0.5M iron (III) chloride solution ($1cm^3$), was added to 35% perchloric acid ($50cm^3$). After treatment, the t.l.c. plates are heated.

Alternatively, the plates were treated with iodine vapour, or iodine solution in petrol, with this treatment, the best antioxidants appeared as persistent coloured spots.

Spectroscopy. U.v. spectra were recorded between 190–390 nm in 95% ethanol solution. I.r. spectra were recorded and calibrated with reference to the absorptions of polystyrene. The appended letters (s, w, br) refer to the type of signal *i.e.* strong, weak, broad *etc.* N.m.r. spectra were recorded with TMS as an internal standard, and chemical shift values are quoted downfield from TMS. The order of citation in parentheses is as follows: i) number of equivalent nuclei (by integration), ii) multiplicity (s, d, t, m *etc.*), iii) coupling constant *e.g.* ^3J 5Hz, iv) assignment. Where hyperfine splitting occurs, multiplicities are given in the form "td" (triplet of doublets), "dm" (doublet of multiplets) *etc.* and the *J* values are quoted in the order: largest to smallest. Mass spectrometric data are given in the form: peak (relative intensity%), with the assignments (*e.g.* M^+) in descending order of relative intensity. Unless stated otherwise, all data were obtained through electronic ionisation at 70 eV.

Instrumentation

m.p.	Electrothermal Mk II.
g.l.c.	Packard 429.
u.v.	Perkin–Elmer Lamda 3.
i.r.	Perkin–Elmer 197.
	Perkin–Elmer 938 G.
^{1}H n.m.r.	Jeol GX FT 400 (400 MHz).
	Jeol GX FT 270 (270 MHz).
	Varian EM–360 (60 MHz).
	Hitachi Perkin–Elmer R24 (60 MHz).
^{13}C n.m.r.	Jeol GX FT 270 (67.8 MHz)
mass	VG 7070E with 2000 data system.
CHN	Carlo Erba Elemental Analyser model 1106.

5:2 Synthesis of DHII and derivatives

5,10–Dihydroindeno[1,2–*b*]indole (20)

A mixture of 1–indanone (13.21g, 0.1mol), and phenylhydrazine hydrochloride (14.48g, 0.1 mmol), was heated in glacial acetic acid (150cm^3). As the temperature of the solution approached the boiling point, the hydrazine hydrochloride dissolved. Soon after, a brown solid precipitated out of solution. The heat was immediately removed, and the reaction allowed to cool. The solid was filtered off, washed with copious amounts of water, and allowed to dry on a sinter. The solid was added to absolute ethanol (150cm^3), heated to boiling, and filtered hot. The product was washed with cold ethanol (150cm^3), and dried in a vacuum oven to yield a beige solid (18.50g, 90%), m.p. 258–9°C (dec) (from chloroform). (Lit.[2] 235°C [dec.], and[3] 245°C [dec]). (Found: C 87.8, H 5.35, N 6.85, Calc. for $C_{15}H_{11}N$: C 87.8, H 5.4, N 6.8%). ν_{max} (Nujol): 3400 cm^{-1}. δ_H (DMSO–d_6): 3.67 (2H, s, 10–H), 7.07 (1H, ddd, 8–H), 7.14 (1H, ddd, 7–H), 7.20 (1H, ddd, 2–H), 7.36 (1H, dd, 3–H), 7.51 (1H, d, 1–H), 7.52 (1H, d, 6–H), 7.57 (1H, d, 9–H), 7.67 (1H, d, 4–H), 11.6 (1H, br, N–H). δ_C (DMSO–d_6): 30.0 (t, C–10), 112.6 (d, C–6), 117.9 (d, C–4) 118.7 (d, C–9), 119.5 (d, C–8), 120.0 (s, C–9b), 121.2 (d, C–7), 124.3 (s, C–9a), 124.7 (d, C–2), 125.6 (d, C–1), 126.8 (d, C–3), 135.3 (s, C–4a), 140.9 (s, C–5a), 143.7 (s, C–4b), 147.8 (s, C–10a). m/z: 205 (100%, M$^+$), 204 (73), 102 (22, [M–1]$^{++}$), 103 (20), 206 (16).

5,10–Dihydro–5–methylindeno[1,2–*b*]indole (66)

Sodium hydride (375mg, 15.6mmol) was added to DMSO (13cm^3) under an atmosphere of nitrogen. The solution was then heated to 70°C until no more gas (H_2) evolved. The solution was cooled to room temperature and 5,10–dihydroindeno[1,2–*b*]indole (2.69g, 13.1mmol) dissolved in a minimum amount of

DMSO was added. After stirring at room temperature for 1 hour, dimethyl sulphate (1.5cm^3, 15mmol) was introduced, and stirring continued for a further 1 hour. Water (3cm^3) was cautiously added, and the reaction then poured into ice/water. The solid thus formed was collected by suction filtration, washed firstly with water, dried on the water pump, and then washed with petrol (60–80°C). Recrystallisation from ethanol yielded colourless needles (1.52g 53%), m.p. 152°C (lit.[4] 153.5). (Found: C 87.5, H 6.0, N 6.4. Calc. for $C_{16}H_{13}N$: C 87.65, H 5.95, N 6.4). ν_{max} (Nujol): 1600 cm^{-1}. δ_H ($CDCl_3$): 3.55 (2H, s, 10–H), 3.85 (3H, s, N–Me), 6.8–7.4 (8H, m, arom. C–H). m/z: 219 (100%, M$^+$), 218 (67), 220 (16), 203 (14, [M–Me]$^+$), 109 (12, [M–1]$^{++}$), 217 (12).

8-Fluoro-5,10-dihydroindeno[1,2-b]indole (67)

8–Fluoro–DHII was prepared by the Fischer indole synthesis using 1–indanone, and *p*–fluorophenylhydrazine hydrochloride, m.p. 225–227°C (from ethyl acetate/petrol [60–80°C]). ν_{max} (Nujol): 3400cm^3. δ_H (DMSO–d_6): 3.68 (2H, s, 10–H), 6.8–7.7 (8H, m, arom. C–H), 11.56 (1H, br, N–H). m/z: 223 (100%, M$^+$), 222 (85), 111 (18, [M–1]$^{++}$).

5,10-Dihydro-8-methoxyindeno[1,2-b]indole (68)

To a stirred solution of *p*–methoxyphenylhydrazine hydrochloride (3.5g, 20mmol), and 1–indanone (2.35g, 20mmol) in absolute ethanol (80cm^3), was added dropwise triethylamine (2.01cm^3, 20mmol). Stirring was continued until the t.l.c. analysis of the reaction mixture indicated that no starting materials remained (about 1 hour). The solvent was removed, and the yellow residue heated at reflux in a solution of polyphosphonate ester in chloroform (made by boiling phosphorus pentoxide (50g) in chloroform (100cm^3) and ether (50cm^3) for

12 hours). After 1 hour, the solvent was removed, and the black residue stirred in water (200cm^3). This mixture was extracted 3 times with diethylether, the organic phases washed with water, and dried ($MgSO_4$). Removal of the solvent yielded a beige solid, which was crystallised from ethyl acetate/petrol (60–80°C) to give beige platelets (3.8g, 78%) m.p. 207°C (lit.[5] 206°C), (Found: C 81.4, H 5.6, N 5.8; Calc. for $C_{16}H_{13}NO$: C 81.6, H 5.6, N 5.95%). ν_{max} (Nujol): 3410cm^{-1} (N–H). δ_H (DMSO–d_6): 3.66 (2H, s, 10–H), 3.79 (3H, s, 8–OMe), 7.4–7.6 (7H, m, arom. C–H), 11.4 (1H, br, N–H). δ_C (DMSO–d_6): 29.7 (q, 8–OMe), 55.2 (t, C–10), 100.5, 110.8, 112.9, 117.6, 124.4, 125.4, 126.5 (d, arom. C–H), 119.6, 135.1, 135.7, 144.1, 147.3, 153.5 (s, quaternary). m/z: 235 (100%, M^+), 168 (52,$[M+1]^{++}$), 192 (26), 211 (26, $[M-Me]^+$), 234 (19), 236 (18).

*6-Chloro-5,10-dihydoindeno[1,2-*b*]indole* (69)

i) The *o*–chlorophenylhydrazone of 1–indanone (m.p. 128°C, 72mg, 0.28mmol) was absorbed onto silica (Merck No. 7736, 500mg) from dichloromethane. The powder was heated to 140°C under a water aspirated vacuum for 30 minutes. On cooling, the product was purified by elution through a pad of "flash" silica with 5% ethyl acetate/petrol (60–80°C), to give a colourless solid (47mg, 65%).

ii) The *o*–chlorophenylhydrazone of 1–indanone (650mg, 2.5mmol) was boiled in a chloroform solution of polyphosphonate ester (see the preparation of dihydro–8–methoxyindeno[1,2–*b*]indole) for 30 minutes. The solvent was removed, and the residue stirred in water (75cm^3) for 1 hour. Extraction into diethylether gave a green solution which was washed with water, dried ($MgSO_4$), and evaporated. Purification by column chromatography (R_f [5% EtOAc/petrol (60–80°C)] 0.5), yielded a colourless solid (500mg, 82%) m.p. 139°C. (Found: C 75.3, H 5.6, N 4.3, $C_{15}H_{10}ClN$ requires: C 75.2, H 5.8, N 4.2%). ν_{max} (Nujol): 3460cm^{-1} (N–H). δ_H

($CDCl_3$): 3.72 (2H, s, 10–H), 7.0–7.6 (7H, m, arom.), 8.5 (1H, br, N–H). δ_C ($CDCl_3$): 30.3 (t, C–10), 117.5, 117.7, 120.8, 120.9, 125.2, 125.5, 126.7 (d, arom. C–H) 117.0, 122.4, 126.1, 134.5, 137.5, 143.8, 147.7 (s, quaternary). m/z: 239 (100%, M^+), 204 (57, $[M-Cl]^+$), 238 (42), 241 (36), 240 (29), 102 (25, $[M-Cl]^{++}$), 203 (21).

*5,10-Dihydro-10-methylindeno[1,2-*b*]indole* (71)

i) *via* 5,10–dihydroindeno[1,2–*b*]indole

5,10–Dihydroindeno[1,2–*b*]indol–10–one (64)

n–Butyllithium (2eq of 1.6M solution) was added to a solution of 5,10–dihydroindeno[1,2–*b*]indole (422mg, 2.1mmol) in dry THF (20cm^3), at –78°C. The solution was warmed to –10°C, and dry oxygen gas bubbled through the blood–red coloured solution for 2 hours. Benzophenone (0.4g, 1eq) was added, and the solution stirred for a further hour. The solution was quenched with a saturated solution of ammonium chloride, and the organic phase collected. Drying ($MgSO_4$), and evaporation of the solvent *in vacuo* yielded after column chromatography (20% EtOAc/petrol [60/80]), the ketone (6, 36mg, 8%), m.p. >250°C (lit.[6] 333–336°C). ν_{max} (Nujol): 3310 (N–H), 1650 (s, C=O). δ_H (DMSO–d_6): 7.0–8.0 (m). m/z: 219 (100%, M^+), 220 (18).

ii) *via* 3–methyl–1–indanone (74)

3–Phenylbutyric acid (75)

A solution of crotonic acid (10.0g, 0.126mol) in dry benzene (175cm^3), was heated to reflux with a Dean–Stark aparatus for 1 hour to remove any final traces

of water. After cooling to 0°C, anhydrous aluminium chloride (30g, 0.23mol) was added portionwise over 30 minutes. Stirring was continued for 10 hours at room temperature. The solution was poured carefully into ice–cold 2M hydrochloric acid (175cm^3) and stirred for 30 minutes. The organic material was extracted into ethyl acetate, and the extract was washed with water, and then dried ($MgSO_4$). The solvents were removed *in vacuo*, and the residue washed through a pad of celite with ethyl acetate (to remove insoluble impurities). The solvent was removed *in vacuo*, and the product crystallised from petrol (60–80°C) to yield a colourless solid (15.0g, 79%) m.p. 35°C Mixed m.p. 34°C (lit.[7] 37°C). ν_{max} (Nujol): 3000 (br, -COOH), 1700cm^{-1} (s, C=O).

3–Methyl–1–indanone (74)

Polyphosphoric acid (125g) was added to 3–phenylbutyric acid (15g, 91mmol), and heated to 150°C, with occasional swirling for 2 hours. Whilst still hot, the red solution was poured into ice/water (300cm^3) and stirred for a further 2 hours. The solution was extracted with ethyl acetate, and the extract was washed twice with 2M sodium hydroxide, water, and then dried ($MgSO_4$). The solvent was removed *in vacuo*, and the residue vacuum distilled to yield a clear liquid (5.1g, 39%) b.p. 144°C (30mmHg) (lit.[8] 120°C [8mmHg]). ν_{max} (liq. film): 1700cm^{-1} (C=O). δ_H ($CDCl_3$): 1.40 (3H, d, ^3J 7.1Hz, 3–Me), 2.26 (1H, dd, ^2J 19.1Hz, ^3J 3.7Hz, 2–H*cis*), 2.93 (1H, dd, ^2J 19.1Hz, ^3J 7.5Hz, 2–H*trans*), 3.43 (1H, ddt, ^3J 7.5, 7.1, 3.7Hz, 3–H), 7.3–7.7 (4H, m, 4–7–H). δ_C ($CDCl_3$): 21.2 (q, C–Me), 32.6 (d, C–3), 45.2 (t, C–2), 123.2, 125.1, 127.2, 134.6 (d, arom. C–H), 136.2, 159.8 (s, C–3a and C–7a), 206.2 (C–1). m/z: 131 (100%, $[M-Me]^+$), 146 (84, M^+), 103 (29, $[M-COMe]^+$), 117 (24, $[M-CO]^+$), 77 (20, $[C_6H_5]^+$), 51 (18, $[C_4H_3]^+$).

*5,10-Dihydro-10-methylindeno[1,2-*b*]indole* (71)

3-Methyl-1-indanone (500mg, 3.42mmol) and phenylhydrazine (0.35cm^3, 3.5mmol), were heated to reflux in glacial acetic acid (20cm^3). After 2 minutes, concentrated hydrochloric acid (1cm^3) was added down the reflux condenser. Boiling was continued for 75 minutes, and then the reaction was cooled. The solution was poured into ice/water, and extracted into ethyl acetate. The extracts were washed consecutively with brine and then water, and dried ($MgSO_4$). Evaporation of solvent *in vacuo*, and column chromatography of the residue (10% EtOAc/petrol [60–80°C]) yielded a cream solid (R_f [30% EtOAc/petrol] 0.7), (320mg, 43%) m.p. 153–155°C (from $CHCl_3$). (Found: C 87.6, H 5.9, N 6.2; $C_{16}H_{13}N$ requires: C 87.6, H 6.0, N 6.4%). λ_{max} (EtOH): 246 (ϵ 18701), 324.5nm (20111). ν_{max} (Nujol): 3420cm^{-1} (N–H). δ_H (DMSO-d_6): 1.50 (3H, d, ^3J 7.3Hz, 10–Me), 3.85 (1H, q, ^3J 7.3Hz, 10–H), 7.0–7.6 (8H, m, arom. C–H), 11.55 (1H, s, N–H). δ_C (DMSO-d_6): 17.8 (q, C–Me), 36.8 (d, C–10), 112.5, 117.8, 118.4, 119.4, 121.0, 124.3, 125.9, 126.8 (d, Arom. C–H), 123.9, 125.8, 134.4, 140.8, 142.1, 153.5 (s, quaternary). m/z: 204 (100%, [M–Me]$^+$), 219 (52, M$^+$).

5:3 Chemistry of DHII

5:3:1 *Attempted oxidation of diindolylmethane*

Preparation of chloro[5,10,15,20–tetraphenyl–21*H*,23*H*–porphinato(2–)–N^{21}, N^{22}, N^{23}, N^{24}]–manganese(III).

A solution of pyrrole (3.5cm^3) and benzaldehyde (5.1cm^3) in propionic acid (250cm^3), was heated to reflux for 30 minutes, and then cooled overnight. The precipitate thus formed was collected by vacuum filtration, washed with water and acetone, and dried in a vacuum dessicator, to yield the porphyrin (1.23g).

The porphyrin (1.55g, 2.5mmol) was added in one portion to DMF (200cm^3) at refluxing temperature, followed one minute later by manganese(II)acetate tetrahydrate (0.62g, 2.5mmol). Heating was continued for a further 10 minutes, and the green solution then allowed to cool slowly to room temperature. The metallo–porphyrin was precipitated out of solution as the chloride salt, by the addition of a solution of sodium chloride (200cm^3 of 0.2M solution) with stirring. The solid was collected by vacuum filtration, washed with water and acetone, and dried in a vacuum dessicator (1.6g, 91%).

Attempted oxidation of diindolylmethane (3)

3,3'–Diindolylmethane (1.5g) was stirred in ethanol (30cm^3) in the presence of a catalytic oxidation system consisting of the manganese salt of the tetra–phenylporphyrin (0.01g), *N*–methylimidazole (0.22cm^3), and a spatula full of platinum supported on polyvinylpyrrolidone, with an atmosphere of oxygen and hydrogen (1:1). Stirring was continued for a number of days, and the experiment analysed by t.l.c. However, the t.l.c. showed no evidence of any identifiable pro–ducts which could be isolated, and only showed the possible presence of polym–eric material.

5:3:2 *Action of radicals on DHII*

Reaction of dimethylamine cation radical on 5,10–dihydroindeno[1,2–*b*]indole – preparation of *9b,9b'-bis-(10*H-*indeno[1,2*-b*]indole* (95)

5,10–Dihydroindeno[1,2–*b*]indole (1.02g, 5.0mmol) was added to a mixture of glacial acetic acid (50cm^3), sulphuric acid (98%, 150cm^3), and chlorodimethy–lamine (0.33g, 1eq), at 0°C. The solution was stirred, and iron(II)sulphate

heptahydrate (0.34g, 0.25eq) added portionwise. Stirring was continued for 30 minutes, and then the reaction was poured onto ice/water (150cm^3). The solution was neutralised (NaOH), causing a white solid to precipitate out. This was collected by vacuum filtration, and washed with water. Column chromatography of this product (20% EtOAc/petrol [60–80°C]) yielded a colourless solid which was shown to be 9b,9b'-bis-(10H-indeno[1,2-*b*]indole, 95), (205mg, 20%) m.p. >280°C (browns at 220°C). (Found: C 88.3, H 5.15, N 6.72. $C_{30}H_{20}N_2$ requires C 88.2, H 4.95, N 6.86%) ν_{max} (Nujol): 1620–1580cm^{-1} (br, C=N). λ_{max}^{EtOH} (nm, ϵ): 203, 63830; 240, 33190; 248, 34722; 287, 24510; 315, 27063. δ_H (DMSO-d_6): 2.72 (2H, d, ^2J 17.6Hz, 10-H [*outer*]), 3.46 (2H, d, ^2J 17.6Hz, 10-H [*inner*]), 6.8–8.1 (16H, m, arom C-H). m/z: 408 (100%, M$^+$), 409 (30), 407 (29), 204 (19, M^{++}).

5:3:3 *Electrochemistry*

Cyclic voltammetry

In a typical experiment, a solution of the indole (20–30mg) was dissolved in a small amount of a 2% solution of sodium perchlorate in acetonitrile. This was placed in an electrolytic cell containing three electrodes as described in the text. These were connected to a precision potentiostat (MINISTAT, H. B. Thompson and Associates), which was connected in turn to a 16 bit ramp generator. The sample was then scanned over a range of 0–2 volts (w.r.t. the standard calomel electrode), at a rate of 10^0 or 10^{-1} volts per second, at a resolution of 2^{16} steps per 10 volts. The voltammetric plot was made on a graph plotter connected directly to the potentiostat.

5:4 Preparation of THII and derivatives

cis-*4b,5,9b,10-Tetrahydroindeno[1,2-b]indole* (100)

To a suspension of 5,10–dihydroindeno[1,2–*b*]indole (19.16g, 93mmol) in glacial acetic acid (300cm^3) was added portionwise over half an hour, sodium cyanoborohydride (24g, 400mmol). The mixture was stirred for 3 hours, until all the material had dissolved. The solution was poured into ice/water (500cm^3) and stirred for 1 hour to break down the borohydride complex. The clear solution was carefully neutralised with sodium hydroxide causing a white precipitate to form. This was filtered and washed with water until the washings were cyanide free. Drying yielded the product as a white solid (19g, 98%), m.p. 107°C (from ethyl acetate/petrol [60–80°C]). (Found: C 86.5, H 6.25, N 6.75. $C_{15}H_{13}N$ requires: C 86.9, H 6.3, N 6.75%). λ_{max} (EtOH): 206, 243, 297. ν_{max} ($CHCl_3$ solution): 3740 cm^{-1} (N–H), 1600; δ_H ($CDCl_3$): 3.20 (1H, dd, ^2J 16.3Hz,^3J 2.0Hz, 10–H*trans*), 3.51 (1H, dd, ^2J 16.3Hz, ^3J 8.4Hz, 10–H*cis*), 3.99 (1H, br, N–H) 4.18 (1H, ddd, ^3J 8.4, 8.4, 2.0Hz, 9b–H), 5.25 (1H, d, ^3J 8.4Hz, 4b–H), 6.60 (1H, d, ^3J 7.8Hz, 6–H), 6.74 (1H, dd, ^3J 7.4Hz, 8–H), 6.99 (1H, dd, ^3J 7.6Hz, 7–H), 7.15–7.22 (4H, m, 1–4–H), 7.32 (1H, d, ^3J 7.5Hz, 9–H. δ_C ($CDCl_3$): 39.1 (t, C–10), 45.7 (d, C–9b), 67.3 (d, C–4b), 110.3, 119.3, 123.8, 124.4, 125.0, 126.9, 127.7, 127.9 (d, arom. C–H), 132.8, 142.3, 143.9, 149.7 (s, quaternary). m/z: 207 (100%, M^+), 206 (65, $[M-1]^+$), 208 (16, $[M+1]^+$), 102.5 (9, M^{++}).

5,6–Dihydroindeno[2,1–*b*]indole (101)

2–Indanone (5.25g, 39.7mmol) [**Care!** avoid contact with metal] and phenylhydrazine hydrochloride (5.74g, 39.7mmol), were heated at reflux in glacial acetic acid (60cm^3) for 1 hour, and then cooled. The solution was poured into ice/water, and the solid precipitate collected by filtration. After partial

purification by column chromatography (R_f [30% EtOAc/petrol (60–80°C)] 0.6), and crystallisation (charcoal) from ethyl acetate, the product was recrystallised from chloroform to yield colourless needles (0.64g, 8%) m.p. 205°C (dec.) (Lit.[9] 200°C). ν_{max} (Nujol): 3400 (N–H), 1600cm^{-1}. δ_H (DMSO–d_6): 3.65 (2H, s, 5–H), 7.0–7.2 (8H, m, arom. C–H), 10.40 (1H, br, N–H). m/z: 205 (100%, M^+), 204 (69), 88 (22, $[M-29]^{++}$), 102 (19, $[M-1]^{++}$), 206 (17).

cis-*5,5a,6,10b-Tetrahydroindeno[2,1-*b*]indole* (102)

5,6–Dihydroindeno[2,1–*b*]indole (185mg, 0.9mmol) was reacted with sodium cyanoborohydride (310mg, 5mmol), in glacial acetic acid (5cm^3), for six hours. The solution was poured into ice/water, and stirred for one hour. It was then neutralised with sodium hydroxide, and the white solid which formed was collected by filtration, washed with water, dried and purified by "flash" chromatography (10% EtOAc/petrol [60–80°C], R_f [30% EtOAc/petrol (60–80°C)] 0.6) to yield a colourless solid (81mg, 43%) m.p. 85–86°C. (Found: C 87.2, H 6.3, N 6.75; $C_{15}H_{13}N$ requires: C 86.9, H 6.3, N 6.75%). ν_{max} ($CHCl_3$ solution): 3380 (N–H), 1600cm^{-1}. δ_H ($CDCl_3$): 3.09 (1H, dd, ^2J 16.3Hz, ^3J 1.5Hz, 5–H*cis*), 3.33 (1H, dd, ^2J 16.4Hz, ^3J 6.2Hz, 5–H*trans*), 3.45 (1H, br, N–H), 4.74 (1H, d, ^3J 8.5Hz, 10b–H), 4.82 (1H, ddd, ^3J 8.5, 6.2, 1.8Hz, 5a–H), 6.55 (1H, d, ^3J 7.7Hz, 7–H), 6.73 (1H, ddd, ^3J 7.5, 7.5Hz, ^4J 1.1Hz, 9–H), 7.00 (1H, ddd, ^3J 7.5, 7.5Hz, ^4J 1.1Hz, 8–H), 7.1–7.4 (4H, m, 1–4–H). δ_C ($CDCl_3$): 41.1 (t, C–5), 53.7 (d, C–10b), 63.7 (d, C–5a), 109.2, 118.8, 124.0, 124.2, 125.1, 127.0, 127.1, 127.8 (d, arom. C–H), 141.5, 143.2, 150.3 (s, quaternary). m/z: 207 (100%, M^+), 206 (84), 204 (18), 178 (17), 208 (15, $[M+1]^+$), 102.5 (10, M^{++}).

cis–4b,5,9b,10–Tetrahydro–5–methylindeno[1,2–*b*]indole (109)

A flame dried flask was charged with sodium hydride (60mg, 2.5mmol), and THF (5cm^3) protected under an atmosphere of nitrogen. To the stirred suspension was added 4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole (500mg, 2.4mmol) in THF (5cm^3) dropwise. The reaction was stirred for 1 hour, a pink colour developing. Iodomethane (0.2cm^3) was added next, and the solution stirred overnight. Water (5cm^3) was added, and the THF removed *in vacuo*. The colourless solid thus obtained was filtered, and dried in a vacuum desiccator. The product was disolved in 5% ethyl acetate/petrol (60–80°C) and filtered through a pad of flash silica to yield, after evaporation of the solvent *in vacuo*, a colourless solid (450mg, 85%) m.p. 76–77°C (from DCM/petrol) (lit.[10] 79.5–80.0°C). (Found: C 86.9, H 6.8, N 6.35; Calc. for $C_{16}H_{15}N$: C 86.85 H 6.8, N 6.35%). ν_{max} ($CHCl_3$ solution): 1600cm^{-1}. δ_H ($CDCl_3$): 3.0 (3H, s, N=Me), 3.1 (1H, dd, ^2J 16.4Hz, ^3J 5.2Hz, 10–H*trans*), 3.4 (1H, dd, ^2J 16.3Hz, ^3J 9.2Hz, 10–H*cis*), 4.1 (1H, ddd, ^3J 8.8, 9.2, 5.2Hz, 9b–H), 4.9 (1H, d, ^3J 8.8Hz, 4b–H), 6.4 (1H, d, ^3J 7.7Hz, 6–H), 6.7 (1H, dd, ^3J 7.8Hz, 8–H), 7.1–7.5 (6H, m, arom.). δ_C ($CDCl_3$): 33.5 (q, N–Me), 39.9 (t, C–10), 45.6 (d, C–9b), 75.8 (d, C–4b), 106.4, 117.3, 124.1, 125.1, 126.6, 128.0, 128.2 (d, arom. C–H), 132.8, 142.2, 144.2, 152.0 (s, quaternary). m/z: 221 (100%, M$^+$), 220 (60, [M−1]$^+$), 206 (19, [M−Me]$^+$), 222 (15, [M+1]$^+$).

cis–*5-Acetyl-4b,5,9b,10-tetrahydroindeno[1,2-*b*]indole* (112)

To a suspension of sodium hydride (25mg, 1mmol) in THF (3cm^3), under an atmosphere of nitrogen, was added 4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole (190mg, 0.92mmol) in THF (2cm^3) dropwise. After stirring at room temperature for 1 hour, acetyl chloride (75μl, 1mmol) was added, and the reaction stirred overnight. Water (3cm^3) was introduced, and the THF solvent removed *in vacuo*, to afford the title compound as a colourless solid (237mg, 100%) m.p. 153°C (from ethanol). (Found: C 81.9, H 6.1, N 5.6; $C_{17}H_{15}NO$ requires: C 81.9,

H 6.05, N 5.6%). ν_{max} ($CHCl_3$ solution): 1640 (br, N–C=O), 1590cm^{-1}. δ_H ($CDCl_3$), mixture of *E/Z* isomers: 2.49 and 2.60 (3H, s, acetyl), 3.26 (1H, dd, ^2J 18.5, 16.5Hz, 10–H*trans*), 3.52 (1H, m, 10–H*cis*), 4.12 and 4.24 (1H, dd, ^3J 8.4 and 7.7Hz, 9b–H), 5.79 and 6.30 (1H, d, ^3J 7.5 and 8.1Hz, 4b–H), 7.0–7.7 (7½H, m, arom. C–H), 8.07 (½H, d, ^{3}J, 7.9Hz 6–H (*Z* isomer). δ_C ($CDCl_3$), mixture of *E/Z* isomers: 24.1 and 24.2 (q, acetyl), 36.7 and 38.0 (d, C–9b), 42.3 and 44.7 (t, C–10), 68.9 and 69.5 (d, C–4b), 115.0, 118.2, 123.7, 123.8, 124.2, 124.4, 125.3, 126.7, 127.1, 127.6, 127.9, 128.2, 128.8 (d, arom. C–H), 140.7, 141.2 (s, quaternary), 159.4 (s, carbonyl–C). m/z: 207 (100%, $[M-Ac]^+$), 249 (54, M^+), 206 (54), 208 (16).

*Protection of 4b,5,9b,10-tetrahydroindeno[1,2-*b*]indole with the phytyl group*

To a mixture of phytol (0.35cm^3, 1mmol) and *S*–collidine (0.15cm^3 1.1mmol), under nitrogen, was added a solution of lithium chloride (47mg, 1.1mmol) in a minimum amount of DMF. The solution was cooled to 0°C, and mesyl chloride (0.09cm^3, 1.1mmol) added with stirring. Stirring was continued at 0°C for 2 hours, and then the reaction poured onto ice/water. This was extracted with 50:50 ether/petrol (60/80). The organic fractions were mixed, washed with saturated copper nitrate solution (5 times), and dried ($MgSO_4$). The solvents were removed *in vacuo*, and the residue dissolved in THF (1cm^3). This solution was added dropwise to the anion of THII made, as previously described, from sodium hydride (20mg, 0.8mmol), and 4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole (158mg, 0.76mmol) in THF (4cm^3). The reaction was stirred at room temperature overnight. Water (5cm^3) was added, and the THF removed *in vacuo*. The aqueous residue was extracted with ethyl acetate, which was washed with water, and dried ($MgSO_4$). TLC analysis of this solution showed two major product spots (R_f. [10% ethyl acetate/petrol (60–80°C)] 0.75 and 0.80). The solvents were

removed, and the residue flash chromatographed, eluting with 5% ethyl acetate/petrol (60–80°C). The two products eluted together to give (a) 5–phytyl– and (b) 5–*iso*–phytyl–4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole in a ratio of 5:2 (as deduced by NMR). ν_{max} (liquid film): 1650 (w, C=C), 1600cm^{-1} (s). δ_H ($CDCl_3$): (a and b), 0.8–2.2 (32H, m, phytyl alkyl–H), 3.10 (1H, dd, 10–H*trans*), 3.45 (1H, dd, 10–H*cis*), 4.18 (1H, ddd, 9b–H), 5.12 (1H, d, 4b–H), 6.40 (1H, d, 6–H), 6.64 (1H, dd, 8–H), 7.04 (1H, ddd, 7–H), 7.12 (1H, d, 9–H), 7.2–7.5 (4H, m, 1–4–H), (a): 5.32 (1H, t(br), 1'–H), (b): 5.05 (1H, dd, ^3J 10.5Hz, ^2J 1.5Hz), 5.21 (1H, dd, ^3J 17.6Hz, ^2J 1.5Hz), 5.92 (1H, dd, ^3J 17.5, 10.5Hz.). m/z (^{i}bu. C.I.): 71 (100%, $[C_5H_{11}]^+$), 485 (69, M^+), 207 (57, [M–phytyl]$^+$), 486 (43, $[M+1]^+$).

cis–*5–Stearyl–4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole* (116)

A solution of 4b,5,9b,10–tetrahydroindeno[1,2–*b*]indole (0.68g, 3.28mmol) in THF (12cm^3), containing TMEDA (0.5cm^3), under an atmosphere of nitrogen, was cooled to –78°C, and *n*–butyllithium (1.45cm^3 of 2.5M solution) added dropwise. The solution was warmed to room temperature to ensure complete formation of the anion, and then cooled back to –78°C. Stearyl bromide (1.25cm^3, 1.1eq) was added, and the reaction stirred at room temperature overnight. The solution was quenched with a saturated solution of ammonium chloride (15cm^3), and the organic component separated, dried ($MgSO_4$), and evaporated *in vacuo*. "Flash" chromatography (petrol [60–80°C]) yielded a white solid (R_f [10% EtOAc/petrol] 0.9), (1.28g, 85%) m.p. 66°C. (Found: C 86.4, H 10.9, N 3.0; $C_{33}H_{49}N$ requires: C 86.2, H 10.75, N 3.05%). ν_{max} ($CHCl_3$ solution): 1610cm^{-1} (s). δ_H ($CDCl_3$): 0.8–1.8 (35H, m, alkyl–H), 3.09 (1H, dd, ^2J 16.2Hz, ^3J 4.9Hz, 10–H*trans*), 3.33 (2H, t, ^3J 7.7Hz, 1'–H), 3.43 (1H, dd, ^2J 16.2Hz, ^3J 9.4Hz, 10–H*cis*), 4.16 (1H, ddd, ^3J 9.4, 8.6, 4.9Hz, 9b–H), 5.16 (1H, d, ^3J 8.6Hz, 4b–H), 6.33 (1H, d, ^3J 7.9Hz, 6–H), 6.59 (1H, ddd, ^3J 7.5, 7.5Hz, ^4J 0.7Hz, 8–H), 7.02 (1H, ddd, ^3J 7.5, 7.5Hz, ^4J 1.1Hz, 7–H), 7.09 (1H, d, ^3J 7.1Hz, 9–H), 7.15–7.5 (4H, m, 1–4–H). δ_C ($CDCl_3$): 14.1 (q,

C–18'), 22.6, 26.6, 27.2, 29.3, 29.5, 29.7, 31.9, 39.7, 46.3 (t, alkyl), 45.2 (d, C–9b), 72.5 (d, C–4b), 106.2, 116.5, 123.9, 125.1, 125.2, 126.4, 127.8, 127.9 (d, arom. C–H), 132.7, 142.4, 143.8, 150.8 (s, quaternary). m/z: 220 (100%, $[M-C_{17}H_{35}]^+$), 43 (74, $[C_3H_7]^+$), 57 (69, $[C_4H_9]^+$), 41 (54, $[C_3H_5]^+$), 459 (50, M^+), 56 (36), 29 (31, $[C_2H_5]^+$), 55 (29), 149 (26).

cis–*5-Stearoyl-4b,5,9b,10-tetrahydroindeno[1,2-b]indole* (117)

A solution of the anion of THII was prepared as described for 5–octadecyl–THII, using THII (0.88g 4.25mmol). The anion was quenched with stearoyl chloride (1.6cm^3). The usual work–up, with extraction into DCM, gave, on column chromatography (eluting with 7% EtOAc/petrol [60–80°C]), a white solid (R_f [10% EtOAc/petrol] 0.5), (0.85g, 42%) m.p. 94°C (from DCM/petrol). (Found: C 83.0, H 10.1, N 2.95; $C_{33}H_{47}NO$ requires: C 83.65, H 10.0, N 2.95%). ν_{max} ($CHCl_3$): 1720cm^{-1} (C=O). δ_H ($CDCl_3$) (mixture of *E/Z* isomers): 0.7–2.0 (33H, m, alkyl), 2.6–3.0 (2H, 2t, 2'–H), 3.1–3.4 (1H, m, 10–H*trans*), 3.4–3.6 (1H, m, 10–H*cis*), 4.0 and 4.3 (1H, dd, 9b–H), 5.84 and 6.33 (1H, d, 4b–H), 7.0–8.2 (8H, m, arom. C–H). m/z: 207 (100%, $[M-C_{18}H_{35}O]^+$), 43 (33, $[C_3H_7]^+$), 57 (27, $[C_4H_9]^+$), 41 (24), 208 (20), 206 (20), also 473 (10, M^+).

Preparation of substituted indanones

3-(Pyrrolodin-1-yl)indene (125)

A mixture of 1-indanone (10g, 76mmol), pyrrolidine (8cm^3, 96mmol), and *p*-toluenesulphonic acid (50mg), was heated to reflux in dry benzene (100cm^3) in a Dean-Stark azeotropic condenser for 18 hours. The reaction was cooled, concentrated *in vacuo*, and the enamine purified by vacuum distillation as an unstable oil (7.5g, 53%) b.p. 120°C (1.0mmHg), (lit.[8] 142-144°C at 2mmHg).

3,3-Dimethyl-1-indanone (123)

i) From 3-(pyrrolidin-1-yl)indene

A solution of the enamine (2.0g, 10.8mmol) in THF (20cm^3) was stirred at 25°C, and *n*-butyllithium (4.8cm^3 of 2.5M solution, 12mmol) added. After the reaction was stirred for 10 minutes, it was cooled to 0°C, and the anion was quenched with iodomethane (0.78cm^3, 12.5mmol). The solution was warmed to room temperature, and stirred for 5 minutes. More *n*-butyllithium (4.8cm^3) was added, and the reaction heated to reflux for 5 minutes. This anion was quenched with iodomethane at room temperature, and stirred for a further 5 minutes. Water (30cm^3) was added, and the solution stirred under nitrogen overnight. The THF was removed *in vacuo*, and the organic material dissolved in ether. This was washed with hydrochloric acid (x2), water, saturated sodium bicarbonate solution, brine, and finally with water. After the solution was dried (Na_2SO_4), and the solvent removed *in vacuo*, the product was separated from other alkylated 1-indanones by column chromatography.

ii) *Via* Friedel craft acylation of benzene

Anhydrous aluminium chloride (43.7g, 0.33mol) was added portionwise to a stirred solution of dimethylacrylic acid (22g, 0.22mol), in dry benzene ($350cm^3$) at 0°C, over a 30 minute period. The reaction was stirred overnight at room temperature. The dark coloured solution was poured into ice–cold 2M hydrochloric acid ($350cm^3$), and stirred for 30 minutes. The organic phase was diluted with ethyl acetate, and separated. The aqueous layer was further extracted with ethyl acetate, the organic solutions combined, washed with water, and dried ($MgSO_4$). Evaporation of the solvents *in vacuo* yielded a gum which, on trituration with petrol, gave 3–methyl–3–phenylbutyric acid as a colourless solid m.p. 57°C (lit[27] 58–59.5°C). The dried solid was mixed with polyphosphoric acid (150g), and heated to 70°C, with occasional stirring, for 30 minutes. The reaction – still warm – was poured into water ($400cm^3$), and stirred for 30 minutes. The solution was extracted with diethylether, and the extract was washed with sodium hydrogen carbonate, and then water. After drying ($MgSO_4$), and evaporation of solvent *in vacuo*, the product was vacuum distilled as a colourless liquid (20.04g, 57% over two steps) b.p. 120°C (19mmHg) (lit.[8] 115–117°C [18mmHg]). ν_{max} (liq. film): $1700cm^{-1}$ (C=O). δ_H ($CDCl_3$): 1.42 (6H, s, 3–Me), 1.65 (2H, s, 2–H), 7.3–7.8 (4H, m, 4–8–H). δ_C ($CDCl_3$): 29.9 (q, C–3–Me), 38.5 (s, C–3), 52.9 (t, C–2), 123.3, 123.4, 127.3, 134.9 (d, arom. C–H), 148.0, 163.8 (s, C–3a and C–7a), 205.8 (s, C–1). m/z: 145 (100%, $[M-Me]^+$), 119 (90, $[M-COMe]^+$), 91 (44, $[C_7H_7]^+$), 160 (44, M^+), 117 (30), 115 (25).

5,10-Dihydro-10,10-dimethylindeno[1,2-b]indole (72)

A solution of 3,3–dimethyl–1–indanone (20.0g, 0.125mol) and phenylhydrazine ($12.3cm^3$, 0.125mol) in glacial acetic acid ($200cm^3$), was heated to reflux. Concentrated hydrochloric acid ($10cm^3$) was added *via* the condenser, and

refluxing continued for a further 2 hours. The solution was allowed to cool, and then poured into water (500cm^3).The water was extracted with diethylether three times, the combined extracts were washed with brine and water, and dried ($MgSO_4$). The solvent was removed, and petrol (60–80°C) added to the residue. The suspension was heated until boiling, the solid filtered off, and the mother liquor concentrated. On cooling, the title compound crystallised out as colourless needles. Further concentration of the mother liquor, and recrystallisation of the product, including recrystallisation of the original suspension, afforded in total (10.2g, 35%) of the product m.p.160°C. (Found: C 87.5, H 6.4, N 5.85; $C_{17}H_{15}N$ requires: C 87.5, H 6.5, N 6.0%). ν_{max} (Nujol): 3410cm^{-1} (N–H). δ_H ($CDCl_3$): 1.60 (6H, s, 10–Me), 7.1–7.7 (8H, m, arom C–H), 8.16 (1H, s, N–H). δ_C ($CDCl_3$): 25.8 (q, C–10–Me), 43.2 (s, C–10), 112.1, 117.4, 118.4, 120.0, 121.5, 122.4, 125.2, 126.5 (d, arom C–H), 123.6, 131.7, 133.0, 139.9, 140.7, 158.6 (s, quaternary). m/z: 218 (100%, $[M-Me]^+$), 233 (46, M^+), 217 (31), 108.5 (18, $[M-Me]^{++}$), 219 (16), 109 (15).

cis-*4b,5,9b,10-Tetrahydro-10,10-dimethylindeno[1,2-*b*]indole* (121)

5,10–Dihydro–10,10–dimethylindeno[1,2–*b*]indole (1.00g, 4.29mmol) was reacted with sodium cyanoborohydride (1.0g, 16mmol) in glacial acetic acid (20cm^3), for 10 minutes. The solution was poured into water, stirred for 30 minutes, and extracted into diethylether. The organic phase was washed 10 times with water, dried (Na_2SO_4), and the solvent removed *in vacuo*. The residue was dissolved in 5% ethylacetate/petrol (60–80°C) and filtered through a pad of "flash" silica, yielding, on removal of solvent, a gum which solidified on the oil pump, to give a colourless solid (0.98g, 98%) m.p. 57–59°C (from DCM/petrol [60–80°C]). (Found: C 86.75, H 7.3, N 5.9; $C_{17}H_{17}N$ requires: C 86.75, H 7.3, N 5.95%). ν_{max} (melt): 3360cm^{-1} (N–H). δ_H ($CDCl_3$): 1.17 (3H, s, 10–Me*trans*),

1.43 (3H, s, 10–Me*cis*), 3.86 (1H, d, ^3J 8.8Hz, 9b–H), 3.9 (1H, br, N–H), 5.29 (1H, d, ^3J 8.8Hz, 4b–H), 6.59 (1H, d, ^3J 7.7Hz, 6–H), 6.71 (1H, ddd, ^3J 7.3, 7.4Hz, 8–H), 7.02 (1H, ddd, ^3J 7.3Hz, 7–H), 7.2–7.3 (5H, m, 1–4–H, 9–H). δ_C ($CDCl_3$): 27.2 (q, 10–Me), 32.2 (q, 10–Me), 47.5 (s, C–10), 59.1 (d, C–9b), 66.8 (d, C–4b), 110.1, 118.5, 122.8, 124.2, 126.2, 127.1, 127.8, 128.3 (d, arom C–H), 129.5, 142.3, 151.3, 153.0 (s, quaternary). m/z: 235 (100%, M^+), 106 (99), 220 (49, $[M-Me]^+$), 204 (21, $[M-2Me]^+$), 234 (20), 236 (16).

2–Methyl–1–indanone (122)

Anhydrous aluminium chloride (40.4g, 0.3mol) was added portionwise over 30 minutes to a solution of ethylcyclopropane carboxylate (10cm^3, 84mmol), in dry benzene (84cm^3). The black solution was boiled for 18 hours, cooled, and poured into a mixture of ice (100g), and 2M hydrochloric acid (50cm^3). The aqueous layer was separated, saturated with salt, and extracted with diethylether. The combined organic solutions were washed with brine, and a saturated solution of sodium hydrogen carbonate, dried ($MgSO_4$), and concentrated. Vacuum distillation yielded a colourless liquid which turned brown after a couple of days exposure to air and light (7.3g, 60%) b.p. 118–122°C (12mmHg) (lit.[11] 108–110°C [5mmHg]). ν_{max} (liq. film): 1710cm^{-1} (C=O). δ_H ($CDCl_3$): 1.3 (3H, d, ^3J 7.3Hz, 2–Me), 2.5–2.9 (2H, m, 2–H, 3–H*cis*), 3.39 (1H, dd, ^2J 17.8Hz, ^3J 8.7Hz, 3–H*trans*), 7.3–7.8 (4H, m, arom. C–H). δ_C ($CDCl_3$): 16.1 (q, C–2–Me), 34.7 (t, C–3), 41.8 (d, C–2), 123.7, 126.3, 127.1, 134.5 (d, arom. C–H), 136.2, 135.3 (s, C–3a and C–7a), 209.2 (s, C–1). m/z: 131 (100%, $[M-Me]^+$), 146 (79, M^+), 117 (28, $[M-CHO]^+$), 103 (21, $[M-COMe]^+$).

Phenylhydrazone of 2–methyl–1–indanone (132)

A solution of 2-methyl-1-indanone (3.01g, 20.1mmol) and phenylhydrazine (2.1cm^3, 22mmol), in glacial acetic acid (15cm^3), was stirred for 30 minutes, and then poured into ice/water. The solution was neutralised with sodium hydroxide, and extracted into diethylether. The organic extract was dried ($MgSO_4$), and evaporated *in vacuo* to yield a solid. This was crystallised from ethanol to give colourless prisms (2.17g, 47%) m.p. 114°C (dec.) (Lit.[12] 116°C). ν_{max} (Nujol): 1600cm^{-1} (w). δ_H ($CDCl_3$): 1.27 (3H, d, ^3J 7.0Hz, 2-Me), 2.64 (1H, d, ^2J 15.6Hz, 3-H*trans*), 3.2-3.4 (2H, m, 3-H*cis*, 2-H), 6.8-7.8 (10H, m, arom. C-H, N-H). m/z: 236 (100%, M^+), 93 (38, [M-indanone]$^+$), 116 (28, [M-hydraz.-Me]$^+$), 122 (18, [M-hydrazone]$^+$).

cis-*4b,5,9b,10-Tetrahydro-9b-methylindeno[1,2-b]indole* (118)

The hydrazone of 2-methyl-1-indanone (1.44g, 6.1mmol) was heated in diethylene glycol (20cm^3) to near its reflux temperature, until ammonia started to evolve from the air condenser. Heating was continued overnight, or until the ammonia ceased to evolve. The solution was cooled, poured into an equal volume of water, and extracted into diethylether. The ethereal solution was back-extracted with 2M hydrochloric acid, which was made basic with sodium hydroxide, and re-extracted with diethylether. The first extract contained no major products (t.l.c. 30% EtOAc/petrol [60-80°C]). The basic componant contained many products, the least polar of which (R_f [30% EtOAc/petrol] 0.8) was purified by column chromatography (5% EtOAc/petrol) to yield a colourless solid (0.38g, 28%) m.p. 72°C (from petrol). (Found: C 87.0, H 6.8, N 6.25; $C_{16}H_{15}N$ requires: C 87.0, H 6.8, N 6.3%). ν_{max} (melt): 3400 (br, N-H), 1610 (s), 750cm^{-1} (s). δ_H ($CDCl_3$): 1.46 (3H, s, 9b-Me), 3.10 (1H, d, ^2J 16.2Hz, 10-H*trans*), 3.30 (1H, d, ^2J 16.3Hz, 10-H*cis*), 4.05 (1H, s, N-H), 4.69 (1H, s, 4b-H), 6.52 (1H, dd, ^3J 7.7Hz, ^4J 0.55Hz, 6-H), 6.71 (1H, ddd, ^3J 7.3, 7.3Hz, ^4J 0.55, 1.0Hz, 8-H), 6.95

(1H, ddd, ^3J 7.9, 7.3Hz, ^4J 1.3Hz, 7–H), 7.0–7.2 (5H, m, arom. C–H). δ_C ($CDCl_3$): 26.5 (q, C–9b[Me]), 46.5 (t, C–10), 53.3 (s, C–9b), 74.2 (d, C–4b), 110.1, 119.0, 122.8, 123.9, 124.6, 126.8, 127.6, 127.7 (d, arom. C–H), 137.1, 142.3, 144.0, 149.0 (s, quaternary). m/z: 221 (100%, M^+), 220 (52), 130 (20, $[C_9H_8N]^+$), 204 (17, $[M-Me]^+$), 222 (16, $[M+1]^+$), 206 (10).

*9b,10-dihydro-9b-methylindeno[1,2-*b*]indole* (130)

A flame dried flask was charged with a solution of the hydrazone of 2–methyl–1–indanone (1.47g, 6.22mmol) in DCM (30cm^3), followed by phosphorus trichloride (3.4cm^3 of 2.0M solution in DCM). The solution was heated to reflux for 2 hours, cooled, and poured into a saturated solution of sodium hydrogen carbonate. After stirring for 1 hour, the organic material was extracted with more DCM. The basic componants were back–extracted into 2M hydrochloric acid. This aqueous solution was made basic, and re–extracted with DCM. Evaporation of the solvent *in vacuo*, and column chromatography (20% EtOAc/petrol [60–80°C]), gave a clear gum (R_f [10% EtOAc/petrol] 0.1) which could be further purified by bulb to bulb distillation (0.4g, 30%) as an unstable gum b.p. 170°C (0.2mmHg). ν_{max} (liq. film): 1710 (w, C=N), 1600cm^{-1}. δ_H ($CDCl_3$): 1.39 (3H, s, 9b–Me), 2.84 (1H, d, ^2J 14.6Hz, 10–H*trans*), 3.11 (1H, d, ^2J 14.5Hz, 10–H*cis*), 6.4–8.0 (8H, m, arom. C–H). m/z: 218 (100%), 219 (82, M^+), 204 (40, $[M-Me]^+$), 217 (34), 108.5 (20, $[M-1]^{++}$), 219 (14).

cis–*4b,5,9b,10-tetrahydro-4b,9b-dimethylindeno[1,2-*b*]indole* (119)

Methyllithium (1.5ml, 2eq of 1.5m solution in hexanes) was added dropwise at –78°C to a solution of 9b,10–dihydro–9b–methylindeno[1,2–*b*]indole (260mg, 1.19mmol) in THF (10cm^3). After stirring at –78°C for one hour, water (1cm^3)

was added to the dark red solution, and the reaction allowed to warm. On approaching room temperature, the colour of the solution disappeared. The reaction was quenched with saturated ammonium chloride solution ($10cm^3$), the organic phase separated, and dried (Na_2SO_4). Evaporation of solvent, and "flash" chromatography (10% EtOAc/petrol [60–80°C]) gave a colourless gum (R_f [10% EtOAc/petrol (60–80°C)] 0.5) which solidified on the oil pump as a colourless solid (87mg, 31%) m.p. 79°C. (Found: C 86.7, H 7.3, N 6.0; $C_{17}H_{17}N$ requires: C 86.8, H 7.3, N 5.95%). ν_{max} (liq. film): 3400 (br, N–H), $1600cm^{-1}$ (s). δ_H ($CDCl_3$): 1.35 (3H, s, 9b–Me), 1.46 (3H, s, 4b–Me), 3.07 (1H, d, ^2J 15.9Hz, 10–H*trans*), 3.36 (1H, d, ^2J 15.9Hz, 10–H*cis*), 4.27 (1H, br, N–H), 6.53 (1H, d, ^3J 7.8Hz, 6–H), 6.71 (1H, ddd, ^3J 7.3Hz, ^4J 1.1Hz, 8–H), 6.96 (1H, ddd, ^3J 7.7Hz, ^4J 1.5Hz, 7–H), 7.1–7.3 (5H, m, 1–4–H, 9–H). δ_C ($CDCl_3$): 22.3, 22.4 (q, 4b–Me and 9b–Me), 45.3 (t, C–10), 54.8 (s, C–9b), 75.2 (s, C–4b), 109.7, 119.0, 121.9, 123.0, 124.4, 126.9, 127.4, 127.5 (d, arom. C–H), 137.7, 140.8, 148.4, 148.8 (s, quaternary). m/z: 220 (100%, $[M-Me]^+$), 235 (95, M^+), 204 (31, $[M-2Me]^+$), 205 (28), 234 (26), 236 (17).

cis-*4b,5,9b-Trimethyl-4b,5,9b,10-tetrahydroindeno[1,2-*b*]indole* (120)

The preparation of 4b,9b–dimethyl–THII was repeated using 9b–methyl–9b,10–dihydroindeno[1,2–*b*]indole (0.86g, 4.0mmol), THF ($8cm^3$), and methyl lithium ($4.0cm^3$ of 1.4M solution), quenching the anion with iodomethane in place of water. After work–up, the least polar componant was separated by column chromatography (1% EtOAc/petrol [60–80°C]), to give a gum which was further purified by bulb to bulb distillation (0.46g, 46%) b.p. 175°C (0.02mmHg). (High Res. Acc. Mass; found: 249.1520; $C_{18}H_{19}N$ requires 249.1517). ν_{max} (liq. film): $1605cm^{-1}$ (s). δ_H ($CDCl_3$): 1.32 (3H, s, 9b–Me), 1.40 (3H, s, 4b–Me), 2.86 (3H, s, N–Me), 2.94 (1H, d, ^2J 15.9Hz, 10–H*trans*), 3.27 (1H, d, ^2J 15.9Hz, 10–H*cis*),

6.30 (1H, d, ^3J 7.7Hz, 6-H), 6.65 (1H, ddd, ^3J 7.3Hz, 8-H), 7.05 (1H, ddd, ^3J 7.7Hz, 7-H), 7.10 (1H, dd, ^3J 7.3Hz, 9-H), 7.15-7.4 (4H, m, 1-4-H). δ_C ($CDCl_3$): 17.0 (q, C-4b[Me]), 21.1 (q, C-9b[Me]), 28.8 (q, N-Me), 46.2 (t, C-10), 54.9 (s, C-9b), 80.2 (s, C-4b), 105.6, 116.9, 122.1, 123.8, 124.7, 126.2, 127.6, 127.7 (d, arom. C-H), 127.1, 136.3, 142.6, 144.8 (s, quaternary). m/z: 234 (100%, $[M-Me]^+$), 249 (93, M^+), 219 (49, $[M-2Me]^+$), 218 (34), 56 (30), 235 (21), 250 (18), 158 (16), 109.5 (12, $[M-2Me]^{++}$), 108.5 (12).

*5,10-Dihydro-6,8-dimethylindeno[1,2-*b*]indole* (135)

A solution of 2,4-dimethylphenylhydrazine hydrochloride (1.27g, 7.35mmol), and 1-indanone (1g, 1.1eq), in glacial acetic acid (15cm^3), was heated to reflux for 30 minutes. The reaction was cooled, and poured into ice/water (200cm^3). This solution was saturated with salt, and extracted into diethylether. The ethereal solution was dried ($MgSO_4$), and evaporated *in vacuo*. The excess acetic acid was removed by azeotropic distillation *in vacuo* with toluene and petrol (60-80°C), to leave a dark coloured solid. The product was purified first by "suction flash" chromatography, and then recrystallisation from petrol (60-80°C) to yield a colourless solid (R_f [30% EtOAc/petrol] 0.8), (0.53g, 31%) m.p.182°C. (Found: C 87.4, H 6.35, N 5.9; $C_{17}H_{15}N$ requires: C 87.5, H 6.5, N 6.0%). ν_{max} (Nujol): 3415cm^{-1} (N-H). δ_H ($CDCl_3$): 2.42, 2.46 (6H, s, 6-Me and 8-Me), 3.62 (2H, s, 10-H), 6.7-7.6 (6H, m, arom. C-H), 8.05 (1H, br, N-H). δ_C ($CDCl_3$): 16.7, 21.4 (q, 6,8-Me), 30.3 (t, C-10), 116.4, 117.1, 124.2, 124.5, 125.5, 126.4 (d, arom. C-H), 120.7, 122.0, 129.5, 138.5, 143.1, 147.8 (s, quaternary). m/z: 233 (100%, M^+), 232 (35), 218 (34, $[M-Me]^+$), 233 (20), 108.5 (18, $[M-Me-1]^{++}$), 217 (11), 116 (10, $[M-1]^{++}$).

cis-*4b,5,9b,10-Tetrahydro-6,8-dimethylindeno[1,2-*b*]indole* (136)

6,8-Dimethyl-DHII (323mg, 1.38mmol) was reacted with sodium cyanoborohydride (400mg, 5eq) in glacial acetic acid solution (7cm^3) for 30 minutes. The solution was poured into ice/water, and stirred for a further 30 minutes. The aqueous solution was neutralised with sodium hydroxide, and the suspension was extracted into diethylether. The organic extracts were washed with water, dried (Na_2SO_4) and evaporated *in vacuo*. Purification by "suction flash" chromatography, gave a white solid (244mg, 75%) m.p.147°C (from EtOAc/petrol [60-80°C]). (Found: C 86.5, H 7.35, N 5.8; $C_{17}H_{17}N$ requires: C 86.75, H 7.3, N 5.95%). ν_{max} ($CHCl_3$ solution): 3400 (N-H), 1600cm^{-1} (s). δ_H ($CDCl_3$): 2.03 and 2.07 (3H, s, 6-Me and 8-Me), 3.18 (1H, dd, ^2J 16.3Hz, ^3J 2.0Hz, 10-H*trans*), 3.48 (1H, dd, ^2J 16.3Hz, ^3J 8.4Hz, 10-H*cis*), 4.16 (1H, ddd, ^3J 8.4, 8.4, 2.0Hz, 9b-H), 5.24 (1H, d, ^3J 8.4Hz, 4b-H), 6.66 (1H, s, 7-H), 6.84 (1H, s, 9-H), 7.1-7.4 (4H, m, 1-4-H). δ_C ($CDCl_3$): 16.8 and 20.8 (q, 6 and 8-Me), 39.2 (t, C-10), 46.2 (d, C-9b), 67.7 (d, C-4b), 122.5, 123.9, 125.1, 127.0, 127.9, 129.4 (d, arom C-H), 119.7, 129.0, 132.5, 142.4, 144.5, 146.1 (s, quaternary). m/z: 235 (100%, M^+), 234 (37), 220 (24, $[M-Me]^+$), 236 (16).

cis-1,2,3,4,4a,5,9b-Hexahydrocarbazole (carbazoline) (137)

Tetrahydrocarbazole (1.2g, 7.0mmol) was reduced by sodium cyanoborohydride (1g, 16mmol) in glacial acetic acid (35cm^3) for 30 minutes. The solution was poured onto ice/water, stirred for 30 minutes, neutralised with sodium hydroxide, and the solid collected by filtration. This was washed with water, and dried on the water pump, and then in a desiccator, to yield a colourless solid (1.19g, 98%) m.p. (from EtOH) 98°C (lit.[13] 99°C). δ_H ($CDCl_3$): 1.3-1.8 (8H, m, 1-4-H), 3.10 (1H, dd, ^3J 6.6, 6.5Hz, 9b-H), 3.64 (1H, br, N-H), 3.72 (1H, dd, ^3J 6.6, 4.75Hz, 4a-H), 6.6-7.3 (4H, m, 6-9-H). δ_C ($CDCl_3$): 21.7, 22.5, 26.9, 29.2 (t, C-1-4), 40.9 (d, C-9b), 59.6 (d, C-4a), 110.1, 118.7, 123.1, 127.0 (d, C-6-9), 133.0, 150.8

(s, C–5a and C–9a). m/z: 130 (100%, $[M-C_3H_7]^+$), 173 (33, M^+), 131 (20).

1,2,3,4–Tetrahydrocyclopent[*b*]indole (138)

A mixture of cyclopentanone (0.1mol) and phenylhydrazine (0.1mol) was heated gently on a warm water bath, whereupon an exothermic reaction took place, forming the hydrazone. A solution of sulphuric acid in water (*ca.* 1:30, 200cm^3) was then added, and the reaction warmed again on the water bath, causing a red gum to drop out of the solution. This solidified on cooling, and was extracted with petrol (60–80°C) from which the indole crystallised as colourless needles which turned brown on exposure to air and light. M.p. 106°C (Lit.[14] 106°C). δ_H (DMSO–d_6): 2.4–2.9 (6H, m, 1–3–H), 6.9–7.3 (4H, m, arom. C–H), 10.8 (1H, br, N–H).

cis–1,2,3,3a,4,8b–Hexahydrocyclopent[*b*]indole (139)

1,2,3,4–Tetrahydrocyclopent[*b*]indole was reduced in glacial acetic acid with sodium cyanoborohydride (5eq). When the reaction was seen to be complete by t.l.c. it was quenched with ice/water, neutralised with sodium hydroxide, and extracted into ether. After the usual treatment, the project was purified by column chromatography to give the indoline as a gum which solidified on a vacuum manifold m.p. *ca.* 25°C (Lit.[15] 21°C). λ_{max} (liq. film): 3430 (br, N–H), 1600cm^{-1}. δ_H ($CDCl_3$): 1.4–2.0 (6H, m, 1–3–H), 3.7 (1H, br, N–H), 3.76 (1H, ddd, ^3J 6.0, 6.4, 2.5Hz, 8b–H), 4.34 (1H, ddd, ^3J 6.0, 8.7, 2.6Hz, 3a–H), 6.5–7.5 (4H, m, arom. C–H). m/z: 130 (100%, $[M-C_2H_5]^+$), 159 (34, M^+), 131 (18).

cis–*4b,5,9b,10-Tetrahydro-8-methoxyindeno[1,2*-b*]indole* (142)

5,10-Dihydro-8-methoxyindeno[1,2-*b*]indole (770mg, 3.3mmol) was reacted with sodium cyanoborohydride (1.0g, 16mmol), in glacial acetic acid ($17cm^3$) solution. After 30 minutes, the solution was poured into ice/water, stirred for 1 hour, and neutralised with sodium hydroxide. The colourless suspension was extracted into diethylether, the organic layers dried (Na_2SO_4), and concentrated *in vacuo*. The residue was column chromatographed (10% ethyl acetate/petrol [60-80°C]) to yield the product (R_f. [30% EtOAc/petrol] 0.5) as a colourless solid (520mg, 66%) m.p. 101°C (from EtOAc/petrol [60-80°C]). (Found: C 81.0, H 6.35, N 5.9; $C_{16}H_{15}NO$ requires: C 81.0, H 6.4, N 5.9%). ν_{max} ($CHCl_3$ solution): $3330cm^{-1}$ (N-H). δ_H ($CDCl_3$): 3.28 (1H, dd, ^2J 16.1Hz, ^3J 1.9Hz, 10-H*trans*), 3.57 (1H, dd, ^2J 16.1Hz, ^3J 8.4Hz, 10-H*cis*), 3.80 (3H, s, 8-OMe), 3.85 (1H, br, N-H), 4.24 (1H, ddd, ^3J 8.4, 8.4, 1.9Hz, 9b-H), 5.30 (1H, d, ^3J 8.4Hz, 4b-H), 6.6-7.4 (7H, m, arom. C-H). δ_C ($CDCl_3$): 38.8 (t, C-10), 46.3 (d, C-9b), 55.8 (q, 8-OMe), 67.9 (d, C-4b), 110.9, 111.2, 112.9, 123.9, 124.9, 126.9, 127.9 (d, arom. C-H), 134.6, 142.1, 143.5, 144.2, 154.1 (s, quaternary). m/z: 237 (100%, M^+), 222 (65, $[M-Me]^+$), 238 (18).

cis-*4b,5,9b,10-Tetrahydro-8-methoxy-5-methylindeno[1,2-b]indole* (143)

Using the same procedure as for 4b,5,9b,10-tetrahydro-5-methylindeno[1,2-*b*]indole, 8-methoxy-THII (239mg, 1.0 mmol) was methylated with iodomethane, using sodium hydride (25mg, 1.1mmol) as the base, in THF ($2cm^3$). Extraction work-up (into diethylether), and purification by "suction" flash chromatography, yielded a clear gum (158mg, 63%) which solidified after bulb to bulb distillation (180°C at 0.2mmHg), m.p. 72°C, (Found: C 81.6, H 6.8, N 5.55; $C_{17}H_{17}NO$ requires: C 81.25, H 6.8, N 5.55%). ν_{max} (liquid film): $1600cm^{-1}$ (s). δ_H ($CDCl_3$): 2.87 (3H, s, N-Me), 3.03 (1H, dd, ^2J 16.3Hz, ^3J 5.5Hz, 10-H*trans*), 3.36 (1H, dd, ^2J 16.2Hz, ^3J 9.2Hz, 10-H*cis*), 3.70 (3H, s, 8-

OMe), 4.08 (1H, ddd, ^3J 8.6, 9.2, 5.5Hz, 9b–H), 4.80 (1H, d, ^3J 8.6Hz, 4b–H), 6.28 (1H, d, ^3J 8.4Hz, 6–H), 6.61 (1H, dd, ^3J 8.4Hz, ^4J 2.7Hz, 7–H), 6.77 (1H, dd, ^4J 2.7, 0.6Hz, 9–H), 7.1–7.5 (4H, m, 1–4–H). δ_C ($CDCl_3$): 34.7 (q, N–Me), 39.2 (t, C–10), 45.6 (d, C–9b), 55.8 (q, 8–OMe), 76.4 (d, C–4b), 107.0, 111.4, 112.1, 124.7, 125.0, 126.3, 127.9 (d, arom. C–H), 134.0, 142.1, 143.8, 146.4, 152.6 (s, quaternary). m/z: 251 (100%, M^+), 236 (95, $[M–Me]^+$), 237 (19), 252 (17).

References to Chapter 5

1. D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, *Purification of Laboratory Chemicals,* Pergamon, Oxford (1980).

2. J. Hausmann, *Chem. Ber.*, 1889, **22**, 2019.

3. F. S. Kipping, *J. Chem. Soc.*, 1894, **65**, 480.

4. J. W. Armit and R. Robinson, *J. Chem. Soc.*, 1927, 827.

5. V. G. Kempter, M. Schwalba, W. Stoss, and K. Walter, *J. Prakt. Chem.*, 1962, **18**, 39.

6. J. J. Eisch and T. Abraham, *Tetrahedron Lett.*, 1976, **20**, 1647.

7. A.-M. Weidler and G. Bergson, *Acta Chem. Scand.*, 1964, **18**, 148.

8. G. Baddeley and R. Williamson, *J. Chem. Soc.*, 1956, 4647.

9. S. R. Cawley and S. G. P. Plant, *J. Chem. Soc.*, 1922, **121**, 827.

10. A. G. Anastassiou, H. S. Kasma, and D. Hauger, *J. Chem. Soc., Chem. Commun.*, 1981, 647.

11. H. W. Pinnick, S. P. Brown, E. A. Mclean, and L. W. Zoller, *J. Org. Chem.*, 1981, **46**, 3758.

12. W. v. Miller and Rohde, *Chem. Ber.*, 1890, **23**, 1889.

13. W. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, 1924, **125**.

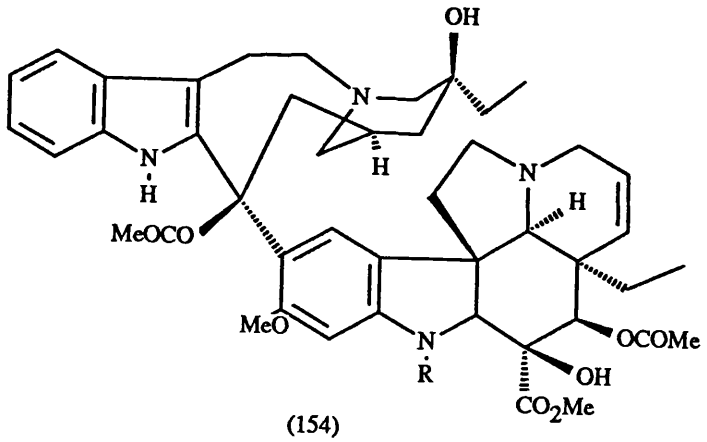
14. W. H. Perkin and S. G. P. Plant, *J. Chem. Soc.*, 1923, 3242.

15. S. G. P. Plant and D. M. L. Rippon, *J. Chem. Soc.*, 1928, 1906.

6 Approaches to Models of the Vinca Alkaloids

6:1 *Introduction*

Vincristine (154, R=CHO) and vinblastine (154, R=Me) are first generation drugs widely used in the treatment of cancer – especially childhood leukaemia. These alkaloids are extracted from plants of the genus *Catharanthus roseus*, formally called *Vinca roseus*, the early name being perpetuated in the name of the drugs. Whilst much research has been undertaken in the synthesis and biosynthesis of these and other related alkaloids, little significant work has been forthcoming on the development of second generation drugs based upon them. Such development has been hampered by a number of factors; for example, the biological profile of vincristine and vinblastine is very complex, the mode of action is still uncertain, and so far little information has been forthcoming from structure–activity studies, which would indicate the precise nature of the pharmacophore. The following account provides a summary of what has been established in this area.



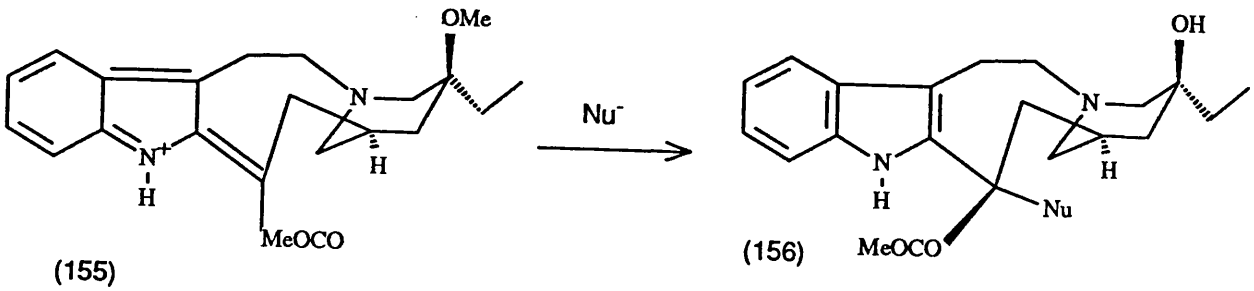
(154)

The alkaloids act at high concentration by inhibiting nucleic acid, and thus protein, synthesis. [1] At low concentration, they promote mitotic arrest leading to

a reduction of cell proliferation, probably by binding to the protein tubulin, thus capping the microtubules which form an integral part of the cytoskeleton of eukaryotic cells.[2, 3]

The only compounds that have been assessed clinically, have been a few structural derivatives of the alkaloids themselves, and these retain the harmful side effects of the parent drugs (causing, for example, alopecia, neuropathy, and nausea). It has always been assumed that both halves of the alkaloids are essential for biological effect, a conclusion that literally doubles the problem presented to the synthetic medicinal chemist, since both halves contain complex functions and stereochemical features.

Recently, however, Magnus has suggested[4] that it is the fragment (155) that is the active unit, acting as a macro–electrophile towards biomolecules in the cell, such as nucleic acids, or proteins, thus leading to adducts of the type (156), (scheme 6:1).



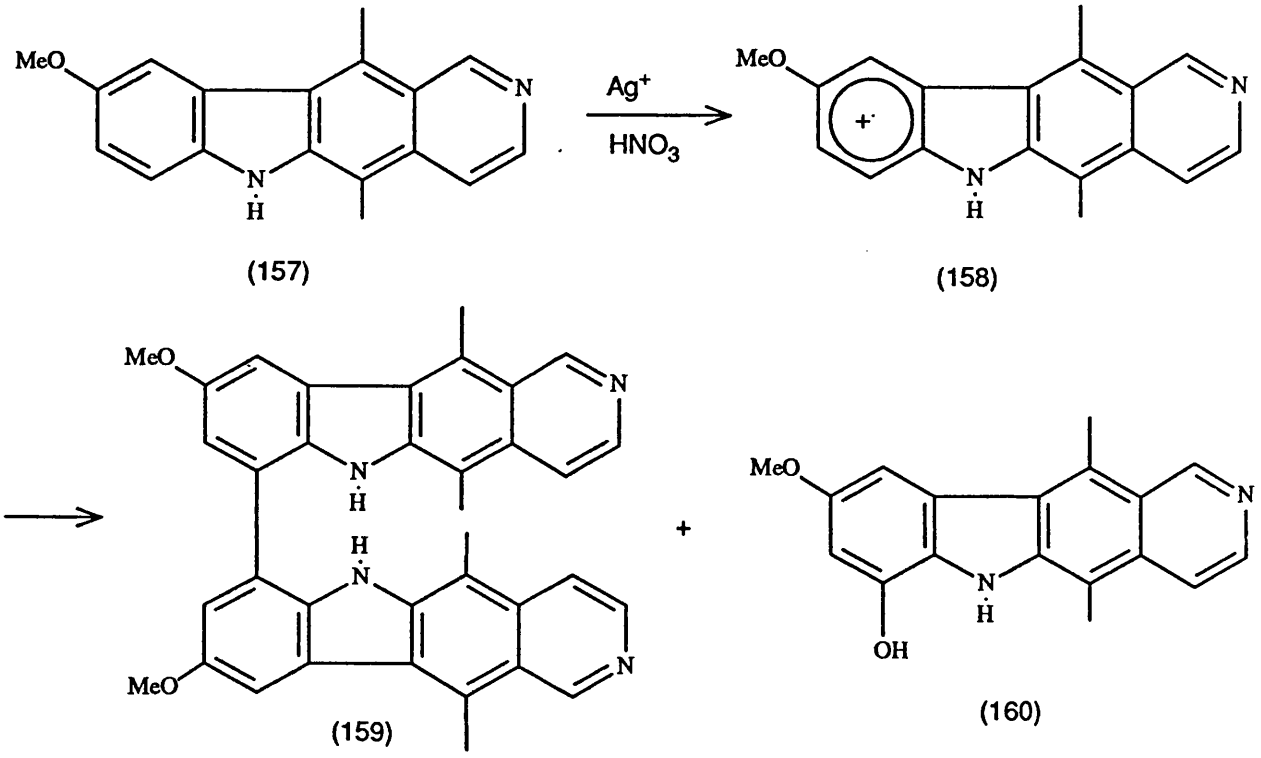
(155) (156)

Scheme 6:1

This is an important hypothesis, as it suggests that the lower portion of the alkaloids acts as a trigger for the activation of the upper portion, and thus at a stroke it paves a way towards the design of simpler analogues which may also be active against cancers.

The question remains, however, as to how the fragmentation process occurs. At Bath we have demonstrated the ease of oxidation of alkyloxylated indole moi–

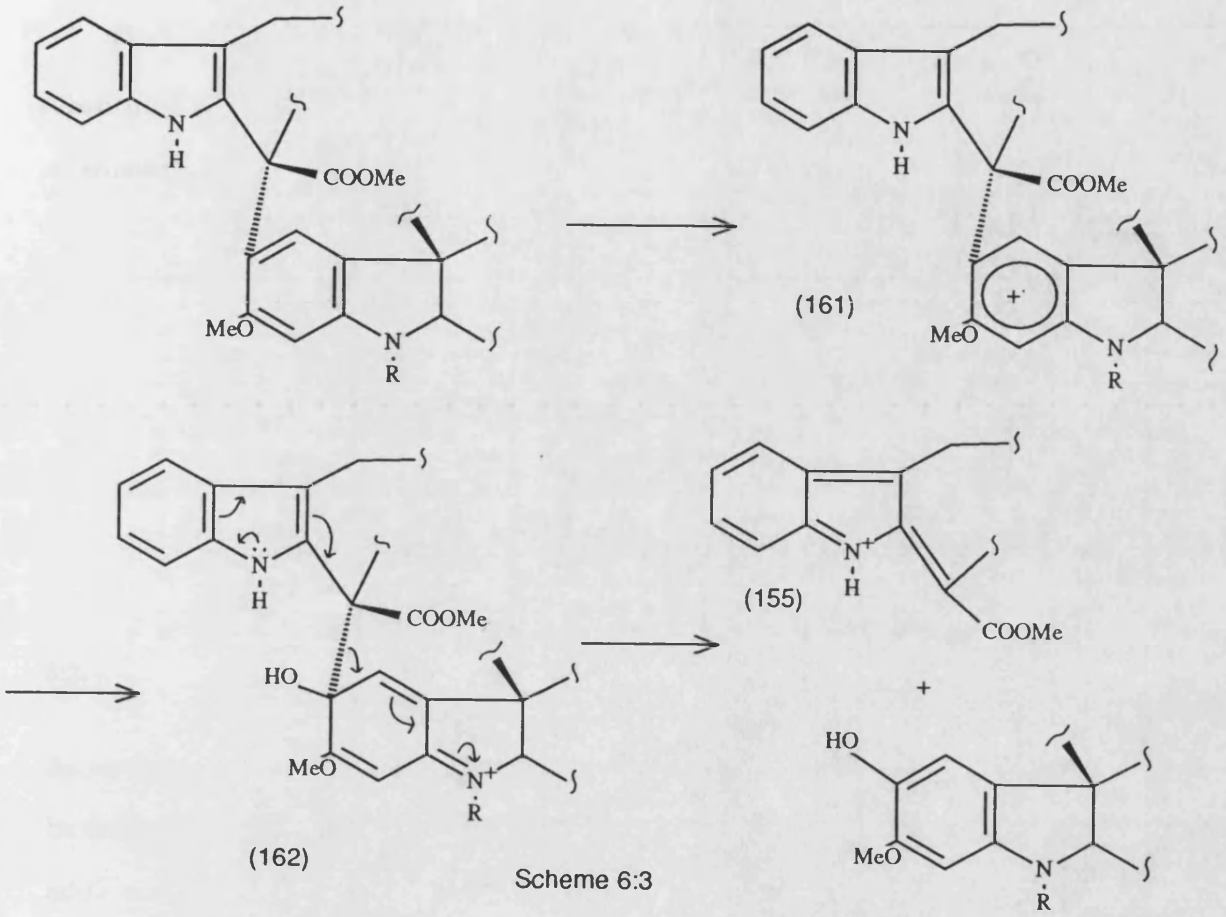
ties in compounds based on the ellipticine alkaloids (and also to a lesser extent in the indenoindoles discussed in chapters 3 and 4). Thus 9–methoxy–ellipticine (157) is readily oxidised to form the radical cation (158) with silver nitrate in dioxan. This species then couples with available nucleophiles such as another molecule of the substrate to yield the dehydrodimer (159), or with water to form the 7–hydroxy derivative (160) (scheme 6:2).



Scheme 6:2

From these observations, we suggest that the *Vinca* alkaloids are activated in a similar fashion. The lower portion of the alkaloid may be oxidised to form the radical cation (161) which may then react with water or another available nucleophile to give an intermediate (162), which then rapidly collapses to give the pharmacophore (155), and the hydroxylated indoline (scheme 6:3). Since this is an oxidative process, the hypothesis may help to explain why the *Vinca* alkaloids are active in oxygenated regions of cancerous tissue, and are ineffective against hypoxic cells which occur in the interfacial region between well–

oxygenated tissue, and necrotic areas.

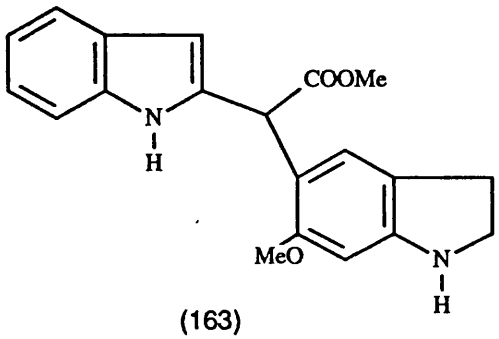


Scheme 6:3

In order to examine this hypothesis, we need therefore to prepare compounds where the structures have been pared down to reveal the essential features for activity. For example, the lower portion of the alkaloids may be reduced to the smallest possible unit to enable the activation of the upper portion. Additionally, it may be possible to reduce the complexity of the upper portion, yet still retain the activity. The author's aim was the development of a route towards simple analogues of the alkaloids in order to examine the feasibility of such radical structural alterations. This work was undertaken at the stage where the study of the indenoindoles looked as though it was terminating. However, the subsequent discovery of the value of the tetrahydroindenoindole series removed all the emphasis from this work, and so this project remains unfinished.

6:2 Discussion of Chemistry

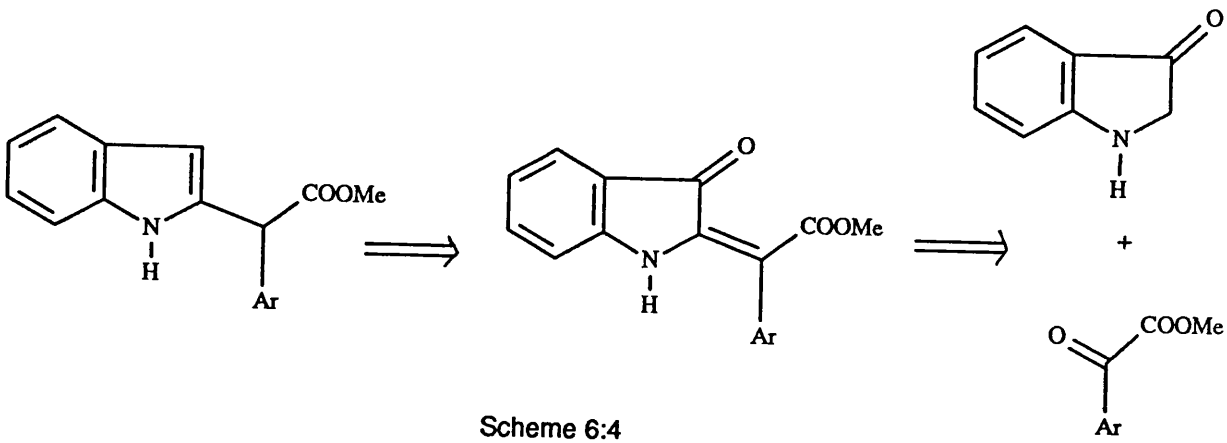
The initial target for study was a structure of the type (163) with an indole substituted at the C–2 by a tertiary carbon bonded to both an ester function and an aromatic "trigger" possessing one or more electron donating species.



(163)

6:2:1 *Retro-synthesis*

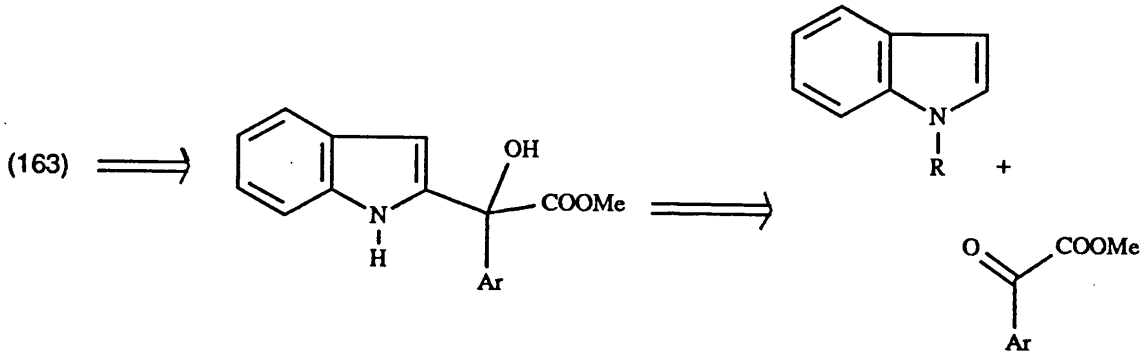
As further functionalisation of the molecule – especially the upper portion – may be desired in the future, we required a synthetic approach that would allow such additions. Therefore two approaches were considered; the first is outlined in scheme 6:4.



Scheme 6:4

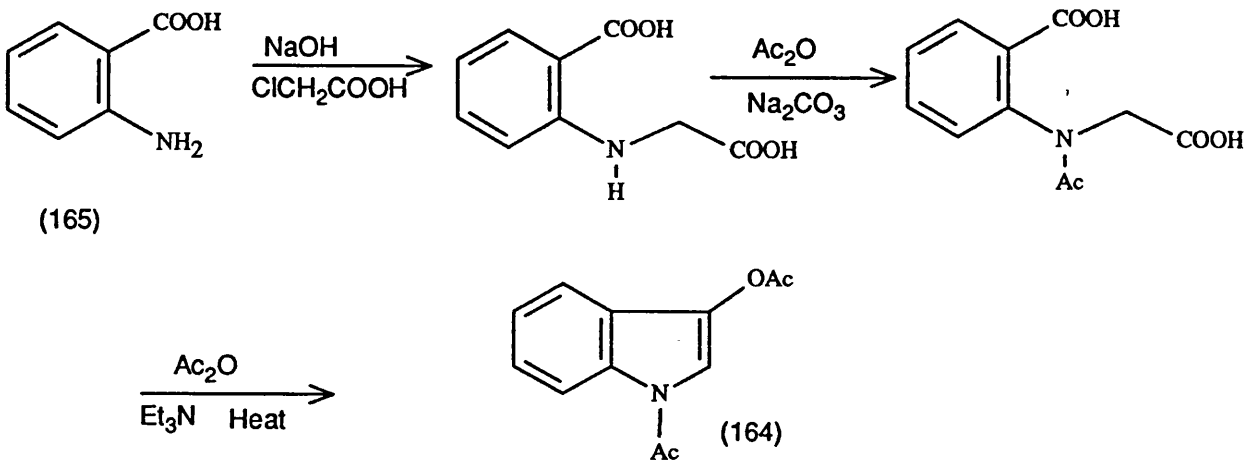
This approach involves the condensation of an α–ketoester with indoxyl in basic solution to give the benzylidene indoxyl derivative. The conjugated ketone could

then be reduced to the 3–hydroxyindole derivative, and dehydrated to the corresponding indole. Alternatively the conjugated ketone could be used in other reactions; for instance a Michael addition to the β–carbon of the enone, or a Wittig reaction directly at the carbonyl group would allow further functionalisation of the molecule. The synthesis of the hydroxyester (163) may also be envisaged *via* substitution of indole at C–2 using a protecting/directing group on nitrogen. The hydroxy function of this hydroxyester should then be readily removed by hydrogenolysis, and further functionalisation is possible through electrophilic attack at the β–position of the indole (scheme 6:5).



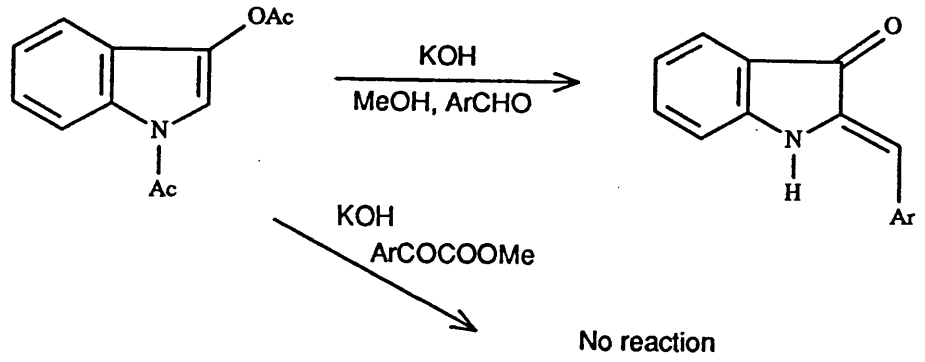
Scheme 6:5

The first of these routes required the preparation of the 1,3–diacetylindoxyl (164)[5] (scheme 6:6). Thus *o*–aminobenzoic acid (165) was alkylated with chloroacetic acid in sodium hydroxide solution. Acetylation of the product *N*–phenylglycine–*o*–carboxylic acid with acetic anhydride in sodium carbonate solution was followed by a ring closure reaction using acetic anhydride and triethylamine at reflux. Liquid/liquid extraction of the crude product, followed by crystallisation from petrol (60–80°C) yielded diacetylindoxyl in an overall yield of 35%. The compound is unstable and readily dimerises and oxidises to give indigotin, if exposed to water and air. Although a model reaction of this product with benzaldehyde in the presence of potassium hydroxide is achieved, leading to the enone (166), a similar condensation with methyl benzoylformate (167) failed



Scheme 6:6

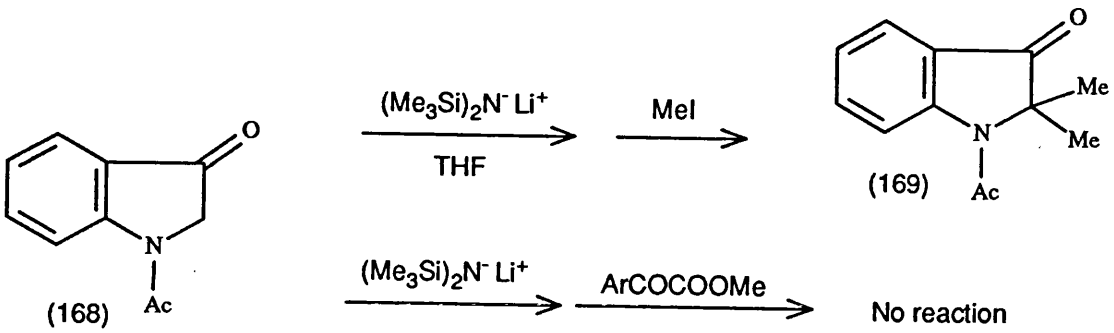
(scheme 6:7).



Scheme 6:7

Obviously, this electrophile is much less reactive than the aldehyde, and in an attempt to enhance the reaction rate, we deliberately set out to form the anion of the indoxyl derivative. In this case, we employed 1–acetylindoxyl (168) instead of 1,3–diacetylindoxyl, and treated it first with lithium hexamethyldisilazane, prior to the addition of the ketoester. Again the reaction failed, although similar alkylations with sterically unencumbered alkylhalides work well. A reaction, for example, with iodomethane and excess base gives 1–acetyl–2,2–dimethylindoxyl (169) (scheme 6:8).

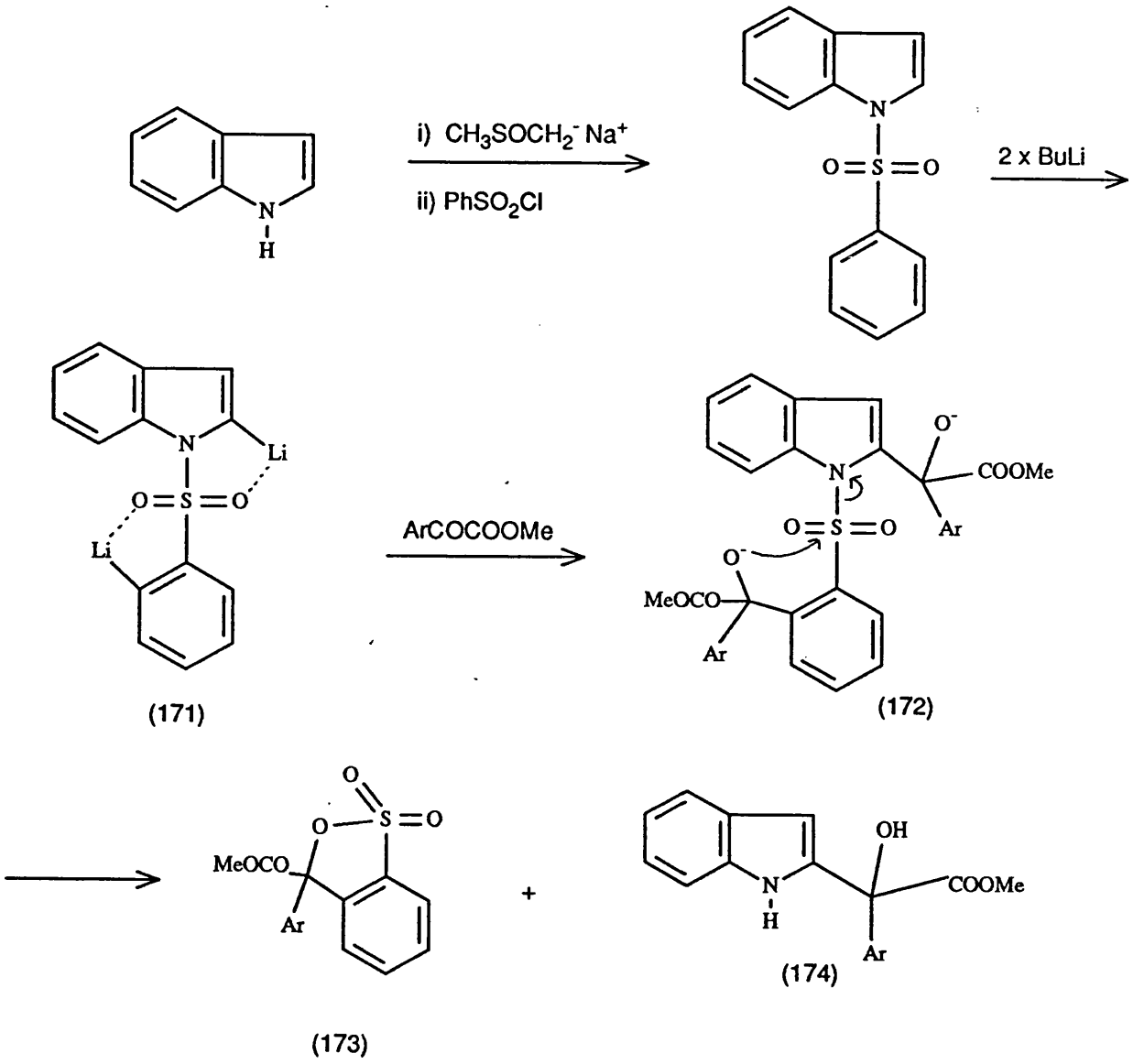
Two techniques for the regioselective 2–deprotonation of indoles are well



Scheme 6:8

established, the first being due to Sundberg.[6] This involves the protection of indole as its *N*–benzenesulphonyl derivative (169), (scheme 6:9); and after deprotonation with a lithium base, the sulphonyl group provides a chelating group for the lithium cation, stabilising thereby the anion formed at the 2 position of the indole. Additionally, deprotonation also occurs readily at the *ortho*–position of the benzenesulphonyl group. The loss of two protons may at first appear problematic, however, as we shall see, with certain electrophiles such as ketones, this feature results in the self–deprotection of the indole, thus removing one step from the synthesis.

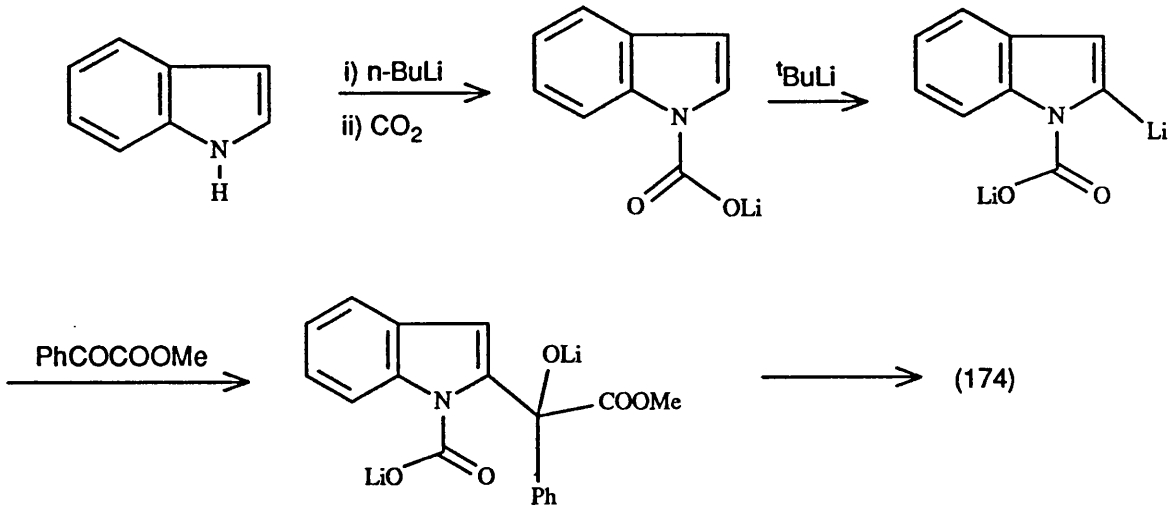
In our hands reaction of *N*–benzenesulphonylindole with two equivalents of *n*–butyllithium, gave the dianion as the chelated dilithium salt (171). In a literature model, this dianion was reacted with methyl benzoylformate to give the adduct (172). Interestingly, the dianion enters into an intramolecular nucleophilic attack (*via* the alkoxy anion) at the sulphur atom of the sulphonamido group, to effect *N*–deprotection as shown (scheme 6:9). In this case then, the products of the reaction are the hydroxyester (174), and the sultone (173). Katritsky has published details of a similar procedure using the lithium indole–1–carboxylate salt as the protection/directing group (scheme 6:10).[7] Two bases are employed in this procedure; *n*–butyllithium is used to acylate indole with carbon dioxide, and this gives the lithium salt of indole–1–carboxylic acid. This is



Scheme 6:9

deprotonated with *tert*-butyllithium to give the dianion (175) which is stabilised through chelation as shown. Reaction with an electrophile results in substitution at the 2-position of the indole, and the carboxylate group is usually removed on work-up. Otherwise the product acid decarboxylates with gentle heating. The advantages of this latter procedure over that of Sundberg, is that an excess of electrophile is not required, and also that the initial step of protecting the nitro-

gen of the indole is more readily achieved.



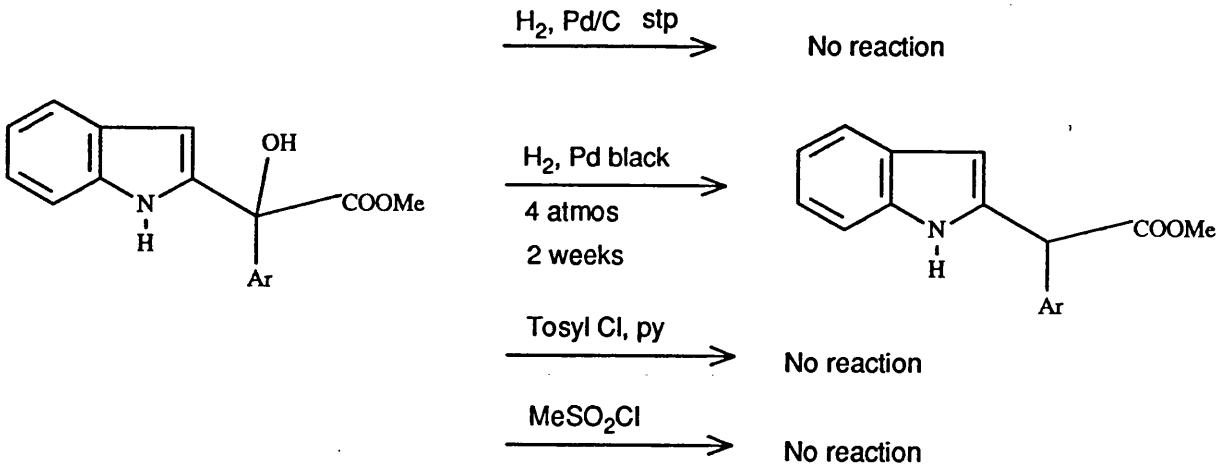
Scheme 6:10

Sundberg's procedure when used with methyl benzoylformate gave the appropriate indole (174) in 65% yield using a six–fold molar excess of electrophile, whereas Katritsky's procedure effected a 50% conversion using only one equivalent.

At this point, we experienced problems in removing the hydroxyl group from the hydroxyester. Direct hydrogenolysis failed to yield more than 4% of the required ester, even though a range of conditions were tried. This indicates poor affiliation between the substrate and the catalyst. The hydroxyester could not be converted into its *p*–toluenesulphonate ester, or methylsulphonate ester (scheme 6:11).

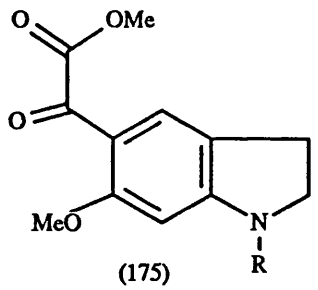
6:2:2 *Synthesis of α-ketoesters*

The failure in these experiments is probably attributable to steric problems, and this did not encourage us to proceed further with this line of research. It was discouraging especially as we had already invested some time in preparing suitable indolinyl–α–ketoesters as reagents towards the desired *Vinca* models. Such



Scheme 6:11

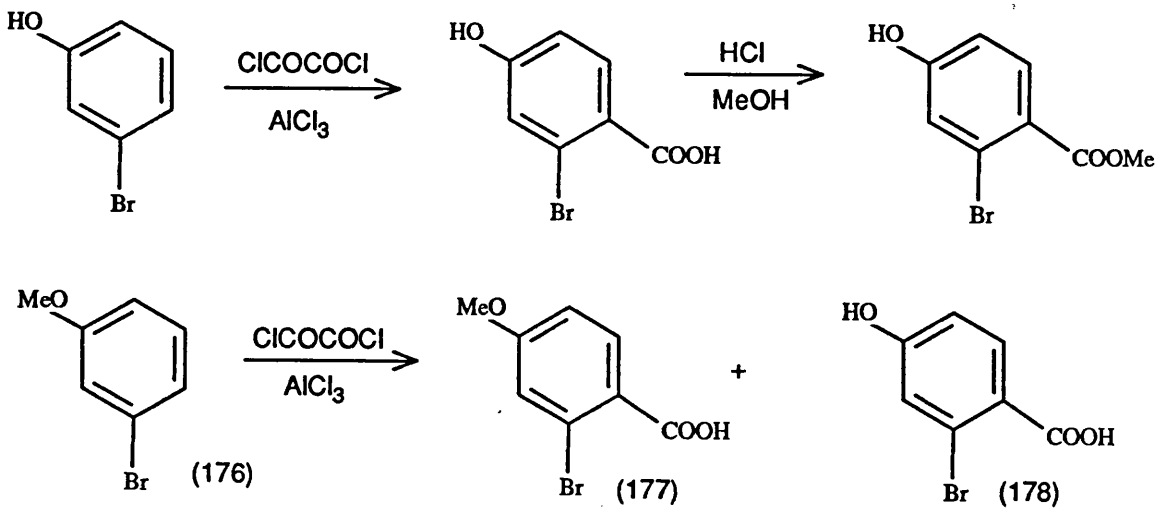
reagents should be of the form (175), and we approached the synthesis of these compounds by first considering the functionalisation of side chains bonded to aromatic units, themselves capable of fabrication into an indoline system.



(175)

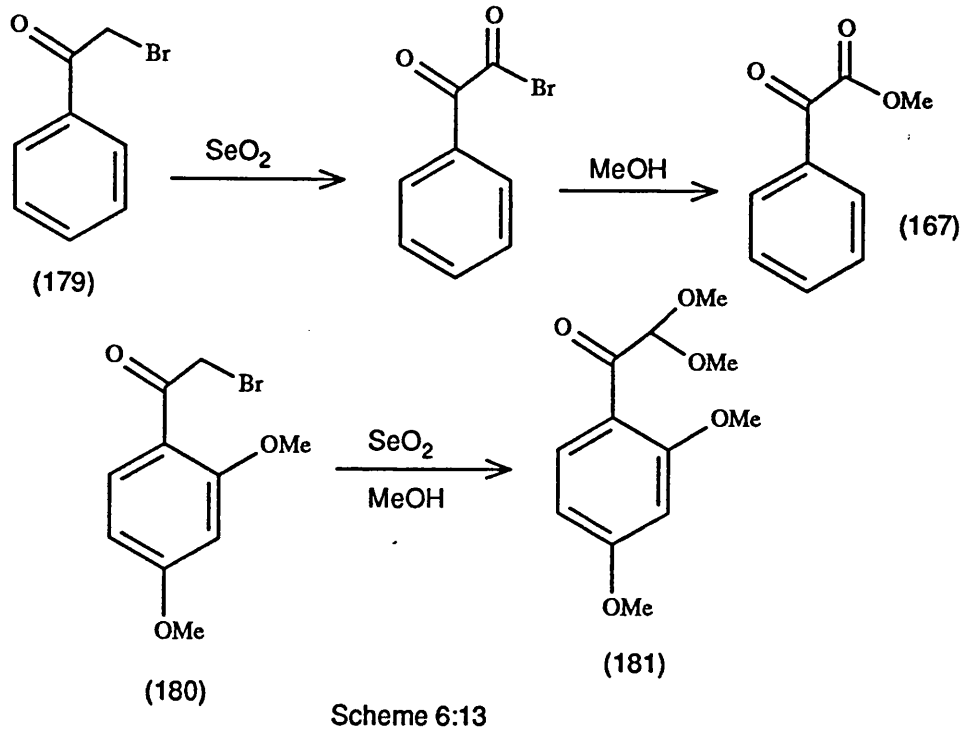
Oxalyl chloride was developed as a replacement for phosgene in the Friedel–Crafts carboxylation of benzene, readily decarbonylating under normal conditions to give the acid rather than the keto-acid. However, Hauser *et al.* have published details of a Friedel–Craft reaction using oxalyl chloride to give a ketoacid.[8] In our hands, repetition of their reaction conditions on various substituted benzene derivatives with hydroxy, methoxy, and bromine substitution gave only normal acids with the expected regiochemistries as shown in scheme 6:12. For further proof of structure, and ease of product separation, the acids were converted into their methyl esters. This was especially necessary in the case of

3-bromoanisole (176), which on acylation, gave a mixture of acids (177) and (178), the latter the *O*-demethylated derivative of the former.



Scheme 6:12

Aryl-α-ketoesters have been prepared by Corey by the selenium dioxide oxidation of phenacylbromide (179) in boiling methanol.[9] Oxidation gives the α-ketoacyl bromide which reacts with solvent to give the ester (167). In this manner, methyl benzoylformate (167) is readily prepared, but when the reaction was repeated with 2,4-dimethoxyphenacylbromide (180), the product isolated was the α-ketodimethylacetal (181) (scheme 6:13). Later on in the series, a similar result was obtained in low yield when the reaction was repeated with a 6-methoxyindoline derivative, thus indicating a general failure of the process for oxidation of substrates bearing an electron rich aryl ring. The mechanism for the normal oxidation reaction is not wholly understood, and there have been two proposals which are shown in scheme 6:14. Corey and Schaefer proposed that the mechanism involves an ene-type reaction of selenous acid with the ketone to give the selenium (IV) ester (182). This rearranges to the selenium (II) ester (183) which on hydrolysis gives the diketone, water, and elemental selenium. Sharpless, however, argues against this proposal[10] stating that selenium (II) esters such as (183) readily hydrolyse to ketols (184). Sharpless proposes that the initial

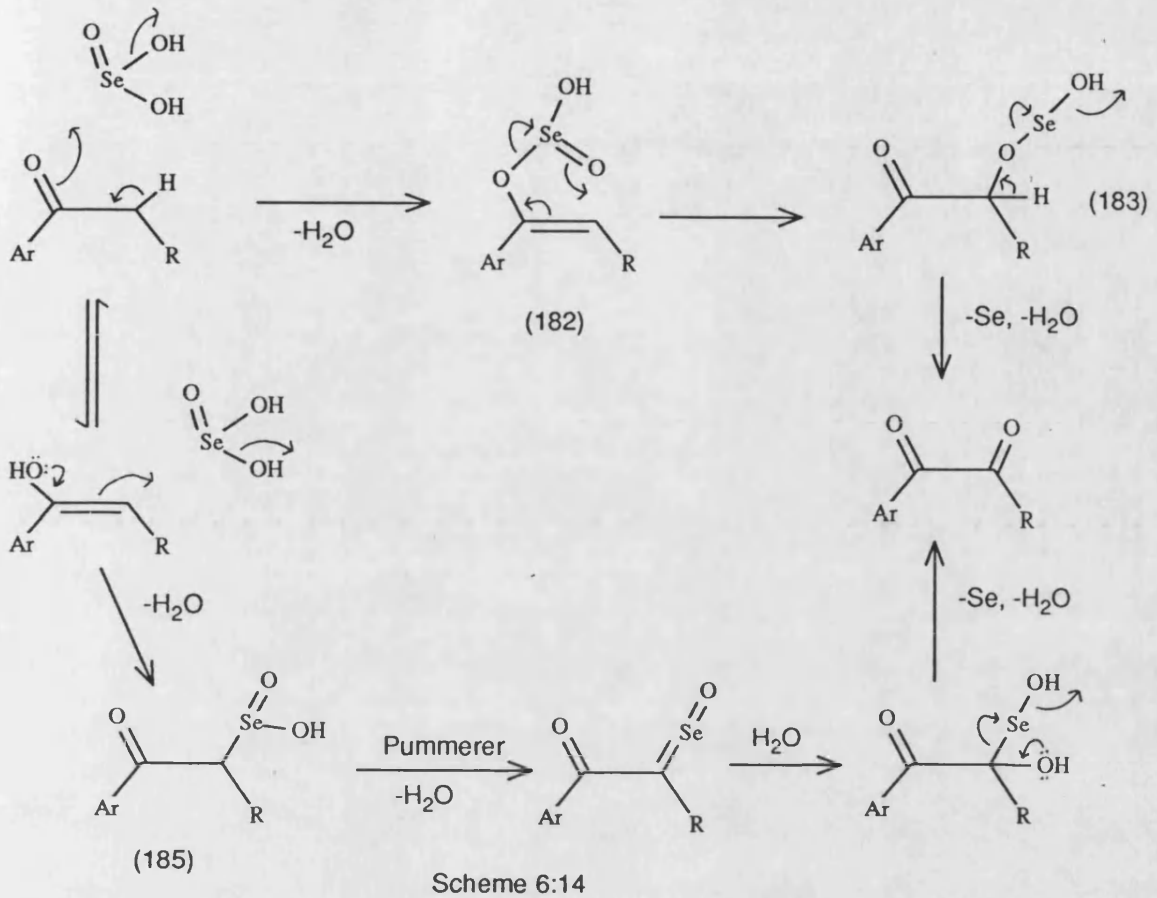


Scheme 6:13

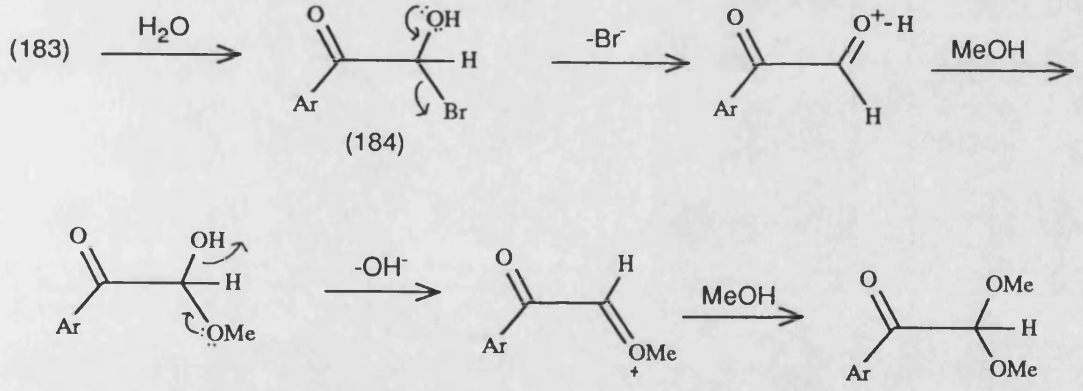
reaction of the selenium dioxide is onto the α–carbon of the enol tautomer of the ketone to give a β–ketoseleninic acid (185). The species undergoes a Pummerer rearrangement and decomposition losing water and selenium metal to yield the α–diketone.

In the case of 2,4–dimethoxyphenacyl bromide, resonance increases the nucleophilicity of the carbonyl oxygen atom, thereby favouring the formation of the selenium (IV) ester. If this intermediate then hydrolyses to the bromohydrin, further reaction with the solvent methanol would afford the observed acetal (181), (scheme 6:15).

With this result still very much in mind, we decided to carry on with the synthesis of the 6–methoxyindoline compounds, and to investigate their substitution reactions under Friedel–Craft conditions. For this, we required 6–methoxyindoline (186). Indoles substituted at the 4 or 6 position are not conveniently prepared by the Fisher process, and there has been much work in



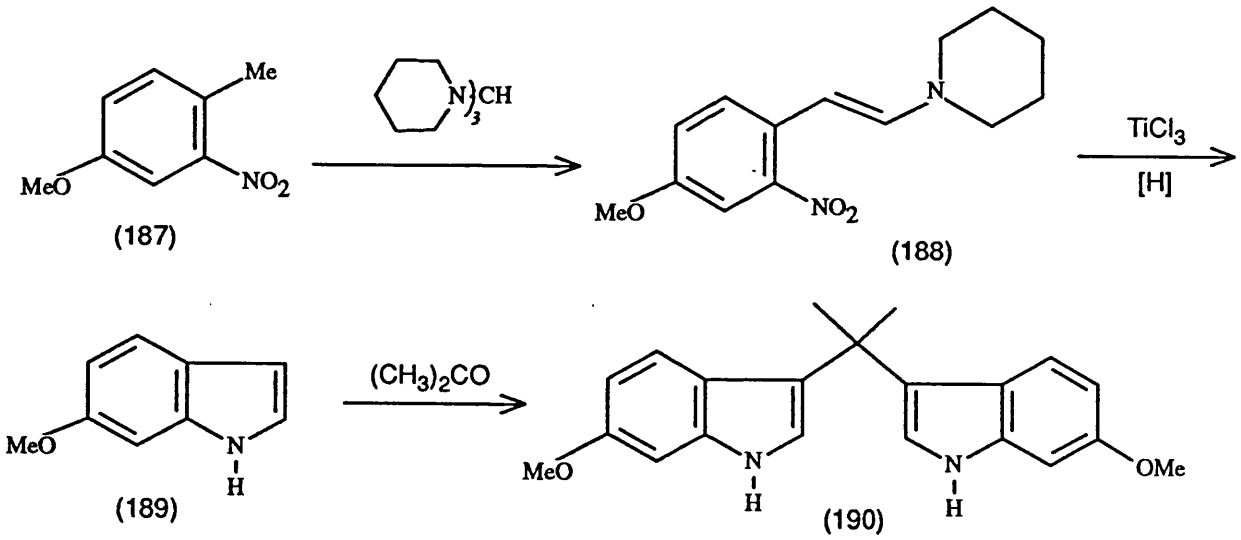
Scheme 6:14



Scheme 6:15

recent years, to devise a satisfactory alternative route. A review of alternative indolisation process has since appeared in the literature,[11] and one method due to Nichols is effective for the synthesis of 6-substituted compounds from the corresponding *o*-nitrotoluenes.[12] In an improvement of the procedure which uses

the dimethylacetal of DMF, 4-methoxy-2-nitrotoluene (187) is condensed with tripiperidinomethane[13] to give the piperidino-2-nitrostyrene (188). This compound may be reductively cyclised by titanium chloride in an ammonium acetate buffer to yield the indole (189). In our hands, this procedure afforded a lowered yield of the desired indole, and a by-product was obtained. A similar by-product was obtained on a different substrate by another member of the group,[14] and we noted from the experimental procedure that acetone is used as a co-solvent. Thus the by-product, which proved to be the *bis*-(indol-3-yl)propane (190) is not at all exceptional (scheme 6:16).

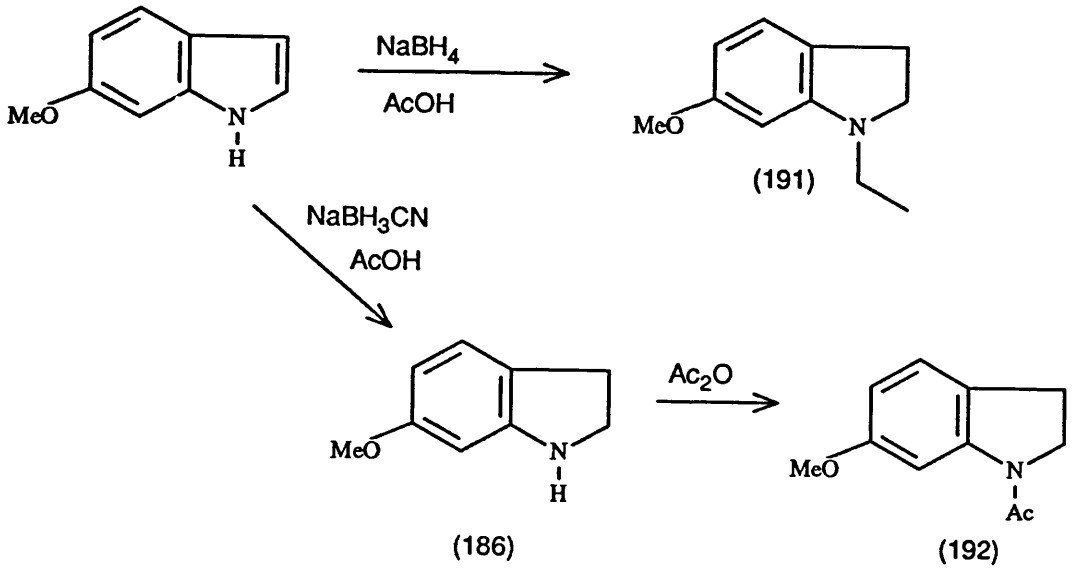


Scheme 6:16

It is surprising that the authors of the original paper had not themselves noticed this point, as we found that a change of solvent from acetone to THF results in a much improved yield.

There are numerous methods for reducing indoles to indolines. Hydrogenation requires strong conditions, and usually results in the reduction of the carbocyclic ring rather than the heterocycle. Zinc in hydrochloric acid works well for 2,3-disubstituted indoles such as THC (28), but for indoles unsubstituted in the pyrrole ring, dimerisation usually results from this process. Gribble has

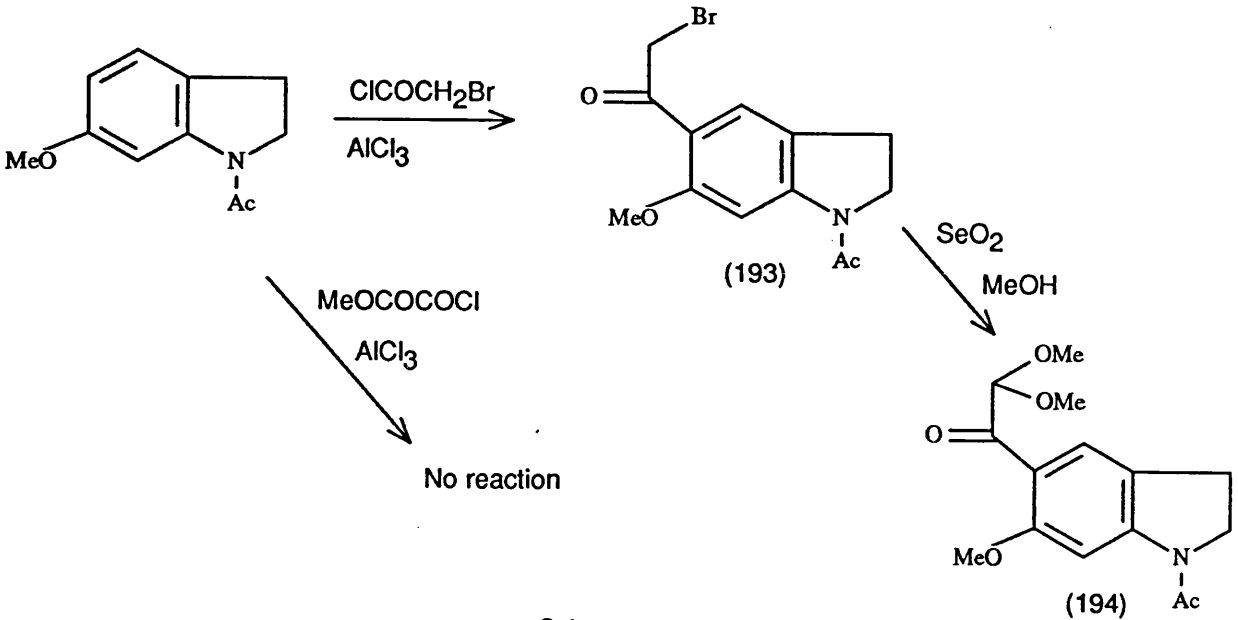
demonstrated the reduction of indole with sodium borohydride in acetic acid, [15] leads to *N*–ethylation of the indoline (scheme 6:17). The mechanism probably involves the participation of an acetoxylated borane species, and the formation of an imine between the reduced acid, and the indoline. This imine is then reduced by further amounts of sodium borohydride, to yield the protected indoline. With 6–methoxy indole, for example, we observe that 1–ethyl–6–methoxyindoline (191) is formed in 85% yield. Gribble states that the use of sodium cyanoborohydride in place of sodium borohydride results in no alkylation, and this observation is confirmed by Kumar and Fluvall, who state that alkylation does occur on warming the reaction to 50°C.[16] This procedure was used to prepare 6–methoxyindoline (186) in 90% yield, which was then acetylated quantitatively to give 1–acetyl–6–methoxyindoline (192) (scheme 6:17).



Scheme 6:17

Acylation of the acetylated indoline with bromoacetylchloride was achieved successfully to give the phenacylbromide derivative (193), but as we mentioned earlier, oxidation of this species with selenium dioxide in methanol, resulted only in the isolation of the dimethyl acetal (194) in low yield (scheme 6:18). Attempted acylation with methyl oxalylchloride gave no reaction (we had hoped

that this would lead directly to the required ketoester).



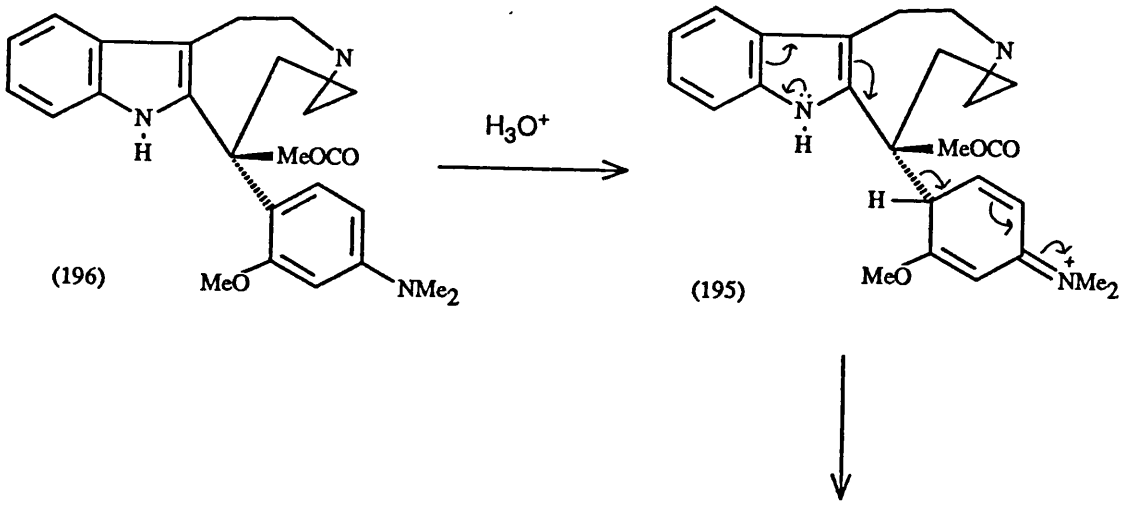
Scheme 6:18

It is obvious that the problems in this part of the study are caused by the effect of the strongly electron donating properties of the methoxy group. The obvious step is therefore to substitute this group for a complementary electron withdrawing function which would be easy to remove at the appropriate point later in the series. The obvious candidate is the tosyl group. Production of the appropriate indoles and indolines was readily achieved using the methods described above. However, before a study of the Friedel–Craft chemistry of the compounds could be implemented, the priority of the indenoindole project overcame the need to continue this project.

6:2:3 *Postscript*

Just as the work described above came to its premature conclusion, a communication appeared in the literature from Magnus on his thoughts on the activation of the *Vinca* alkaloids.[17] Magnus favours *ipso* protonation of the lower half of

the alkaloid to give the iminium ion (195) which then collapses as shown to the pharmacophore which is then available for nucleophilic attack (scheme 6:19). To test the hypothesis, he made a model substrate using as a trigger, 4-dimethylamino-2-methoxyphenyl and and a modified model of velbelamine as the upper half (196). This molecule proved to be weakly cytotoxic, and we wait with interest for any further results from Magnus' laboratory which might confirm whether this approach has value in cancer chemotherapy.



Scheme 6:19

etc. (see scheme 6:3)

6:3 *Experimental for Chapter 6*

For an introduction to experimental details, the reader is directed to page 122.

N–Phenylglycine–*o*–carboxylic acid

Anthranilic acid (50g, 0.365mol) was slurried in water (35cm^3), and dissolved in sodium hydroxide solution (15.4g in 28.5cm^3 water). Separately, sodium carbonate (20g) was added to a solution of chloroacetic acid (34.7g, 0.367mol) in water (50cm^3). The two solutions were heated to 40°C, and mixed. Stirring was continued at 40°C overnight, and the solution cooled to room temperature. When cold, the solid was dissolved in sodium hydroxide solution (400cm^3 of 4% solution), and this solution carefully neutralised with concentrated hydrochloric acid. The resulting precipitate was collected, washed with water, and dried in a vacuum dessicator. Crystallisation from methanol yielded tan needles (60g, 85%) m.p. 218°C (lit.[18] 215°C).

N–Acetyl–*N*–phenylglycine–*o*–carboxylic acid

N–Phenylglycine–*o*–carboxylic acid (12.5g, 64mmol) was added portionwise to a solution of sodium carbonate (10g) in water (150cm^3). After dissolution, acetic anhydride (10cm^3) was added slowly, with evolution of a gas. The brown coloured solution was stirred for 30 minutes, and then acidified with concentrated hydrochloric acid. The suspension thus formed was filtered and dried *in vacuo* to yield a tan coloured solid (6.8g, 45%) m.p. 204°C (lit.[19] 214°C).

N,*O*–Diacetylindoxyl (164)

N–Acetyl–*N*–phenylglycine–*o*–carboxylic acid (6.84g, 28.9mmol) was dissolved in acetic anhydride (50cm^3) and triethylamine (10cm^3). The solution was heated at reflux temperature under an atmosphere of nitrogen for 20 minutes, cooled, and excess solvent removed *in vacuo*. The remaining liquid was extracted in a liquid/liquid extractor, with petrol (60–80°C). The petrol was reduced in volume, and the product allowed to crystallise as pale green needles (3.3g, 52%) which were stored at –20°C under an inert atmosphere: m.p. 77°C (lit. 77–77.5°C). δ_H ($CDCl_3$): 2.35 (3H, s, $OCOCH_3$), 2.6 (3H, s, $NCOCH_3$), 7.3–7.6 (3H, m, 5,6,7–H), 7.7 (1H, s, 2–H), 8.5 (1H, m, 4–H).

Attempted condensation of *N*,*O*–diacetylindoxyl with electrophiles.

i) Potassium hydroxide as base and methyl benzoylformate as electrophile.

A solution of methyl benzoylformate (0.60cm^3, 4mmol) and potassium hydroxide (250mg, 3eq.) in methanol/water (30cm^3) was degassed with a stream of nitrogen for one hour. *N*,*O*–Diacetylindoxyl (300mg, 1.38mmol) was added under nitrogen, and the flask tightly stoppered. No reaction was seen to occur, although the ester slowly dissolved into the basic solution implying that it was being saponified.

ii) Lithium hexamethyldisilazide as base, and iodomethane as electrophile.

A flame–dried flask was charged with hexamethyldisilazane (0.25cm^3), 1.2mmol) and dry ether (10cm^3). *n*–Butyllithium (0.76cm^3 of 1.6M solution) was added dropwise, and the reaction heated to reflux for 30 minutes during which time a white solid appeared on the sides of the flask. The solvent was removed, and the resulting solid dissolved in THF (10cm^3) and cooled to –78°C. A solution of

N,O–diacetylindoxyl (220mg, 1.0mmol) in THF (2cm^3) was added dropwise. The yellow solution was allowed to warm to room temperature, and stirred for 30 minutes. The solution was recooled to –78°C, and iodomethane (0.75cm^3) added. The reaction was allowed to warm to room temperature, and quenched with saturated ammonium chloride. The organic layer was diluted with ethyl acetate, separated and washed twice with brine, dried ($MgSO_4$) and solvent removed *in vacuo*. T.l.c. revealed the presence of many products, the major one being isolated by column chromatography (30% EtOAc/petrol [60–80°C]) as a pink solid, and found to be *N*–acetylindoxyl m.p.136–8°C (for spectral details, see following experiment).

N–Acetylindoxyl (168)

N,N–Diacetylindoxyl (600mg, 2.75mmol) was dissolved in a minimum amount of THF, and added to a solution of sodium tetraborate decahydrate (100ml, 0.025M solution, pH 9.25). After one week, the organic material was extracted into ether, which was washed with water and dried ($MgSO_4$). Removal of solvents *in vacuo*, and column chromatography yielded starting material, and the product which crystallised from EtOAc/petrol (60–80°C) as light tan needles (110mg, 24%) m.p. 136°C (lit.[20] 136°C). ν_{max} ($CHCl_3$ solution): 1720 (s, *N*–$COCH_3$), 1675 (s, C=O). δ_H ($CDCL_3$): 2.05 (3H, s, *N*–Ac), 4.0 (2H, s, 2–H), 6.93 (1H, ddd, 5–H), 7.3–7.5 (2H, m, 4,6–H), 8.25 (1H, d, 7–H). m/z: 133 (100%, $[M-Ac]^+$), 105 (71, $[M-Ac-CO]^+$), 175 (70, M^+), 43 (68, Ac^+), 104 (46), 77 (39, $[C_6H_5^+]$), 132 (25).

Reaction of *N*–acetylindoxyl with lithium hexamethyldisilazide, iodomethane as electrophile.

N–Acetylindoxyl (12.5mg, 0.072mmol) in THF (0.5cm^3) was added to a solution

of lithium hexamethyldisilazide prepared as previously described from hexamethyldisilazane (0.017cm^3, 1.1 eq.), in THF (5cm^3), at -78°C. The reaction was allowed to warm to room temperature, and then cooled again to -78°C, and iodomethane (0.35cm^3) added. The reaction was again allowed to warm to room temperature, and stirred for 1.5 hours, whereupon it turned green in colour. The reaction was quenched with water (0.5cm^3), and the THF removed *in vacuo.* The organic material was isolated by extraction between ethyl acetate and water (x3), and the organic layers collected, dried ($MgSO_4$) and solvents removed *in vacuo.* The major product (R_F 0.3 [30% EtOAc/petrol (60-80°C)]) was purified by column chromatography, and shown to be *1-acetyl-2,2-dimethylindoxyl* (6mg, 41%) indicating insufficient time was allowed for the formation of the anion of the indoxyl derivative. (High res. acc. mass 203.0941; $C_{12}H_{13}NO_2$ requires 203.0946) δ_H ($CDCl_3$): m/z:

1-Benzenesulphonylindole (170)

A flame dried flask was charged, under an atmosphere of nitrogen, with freshly distilled DMSO (25cm^3) and sodium hydride (0.66g, 1.1 eq). The reaction was heated to 70°C until evolution of hydrogen gas ceased, and then cooled to 0°C. Indole (3.2g, 27.25mmol) in diethylether (10cm^3) was added dropwise, and the reaction stirred for 30 minutes and then allowed to warm to room temperature. The reaction was then recooled to 0°C, and benzenesulphonyl chloride (5cm^3, 1.5eq) added dropwise. A creamy coloured suspension developed. After stirring at room temperature for 1 hour, water (5cm^3) was cautiously added, and then the whole mixture was poured into excess water (200cm^3). The organic material was extracted into ether (x5), which was washed with more water, and dried ($MgSO_4$). Removal of solvent *in vacuo* yielded an oil which was triturated with petrol (60-80°C)/ether (2:1) to give a colourless solid which was collected by

filtration, and copiously washed with petrol. The solid was dried to yield the title compound as a colourless solid (2.84g, 42%), m.p. 77°C (lit.[21] 77.5–78°C)

Methyl 2-hydroxy-2-(indol-2-yl)-2-phenylacetate (174)

i) *Via* the procedure due to Sundberg

A solution of 1–benzenesulphonylindole (300mg, 1.17mmol) in THF (5cm^3) was cooled in an ice/salt bath, and tetramethylethylenediamine (0.2cm^3, 1.1eq) added, followed by *n*–butyllithium (1.6cm^3 of 1.6M solution in hexane). The reaction was stirred for one hour, and then cooled to –78°C. The golden coloured solution of the dianion was then added to a solution of methyl benzoylformate (1 cm^3) in THF (10cm^3) at –78°C, and the reaction stirred at this temperature for 2.5 hours. 10%Acetic acid/methanol (3cm^3) was then added, and the reaction warmed slowly to room temperature. The solution was diluted with water (10cm^3), and extracted into DCM. The organic layer was washed with brine, and dried ($MgSO_4$). T.l.c. indicated two major products, one of which (R_F 0.5 [30%EtOAc/petrol(60–80°C)]) gave a coloured spot in a dip of $FeCl_3/HClO_4$, indicating an indole. This was isolated by column chromatography and identified as the title compound (275mg, 84%).

ii) *Via* the procedure of Katritsky.

A flame dried flask was charged under nitrogen, with indole (1g, 8.5mmol) in THF (15cm^3). The solution was cooled to –78°C, and a solution of *n*–butyllithium in hexane (1.1equivalent of 1.6M solution) added. The solution was stirred at –78°C for one hour, during which another flame dried flask was charged with THF (30cm^3), and this saturated with dry carbon dioxide gas. This second solution was cooled to –78°C, and the anion solution added to it. The reaction was

allowed to warm to room temperature, and the solvent removed *in vacuo*. The solid was dissolved in THF ($15cm^3$), and cooled to -70°C. *t*-Butyllithium (1.1 equivalent of 1.6M solution in pentane) was added dropwise, and the reaction stirred at -70°C for 1 hour. The whole solution was then added to a solution of methyl benzoylformate (1 equivalent) in THF ($5CM^3$) at -70°C. The reaction was stirred at -78°C for two hours, and then water ($1cm^3$) added. The reaction was allowed to warm to room temperature, and then was poured into a saturated solution of ammonium acetate ($30cm^3$). The organic phase was diluted with DCM, collected, washed with brine, and dried (Na_2SO_4). Removal of solvent *in vacuo* and purification by column chromatography yielded the title compound as a colourless solid (1.4g, 60%) m.p. 142°C. (Found: C 72.6, H 5.35, N 4.9; Calc. for $C_{17}H_{15}NO_2$: C 72.6, H 5.4, N 5.0%). λ_{max} nm (rel.abs.): 216 (0.86), 256 (0.23), 264 (0.23), 0.16 (290). ν_{max} ($CHCl_3$): 3520 (O-H), 3460 (N-H), 1730cm^{-1} (C=O). δ_H ($CDCl_3$): 3.89 (3H, s, COOMe), 4.41 (1H, br, N-H), 6.61 (1H, d, ^4J 2.0Hz, 3'-H), 7.0-8.0 (8H, m, arom.), 8.5 (1H, br, O-H). m/z (low eV E.I.): 281 (100%, M^+), 222 (61, $[M-COOMe]^+$), 107 (46, $[M-indole-COOMe]^+$), 105 (23), 282 (22), 156 (14, $[M-indole]^+$).

Attempts at removing the hydroxy function in (174).

i) Hydrogenation at atmospheric pressure in methanol over palladium/carbon resulted in 80% recovery of starting material.

ii) Reaction with tosyl chloride in pyridine resulted after five days in 85% recovery of starting material.

iii) Reaction of a small sample with 1 equivalent of triethylsilane in trifluoroacetic acid resulted in the recovery of a very small amount of a colouless solid; M^+ (E.I.) 528 (equivalent to dehydrodimerisation of possible product). This compound was unstable, and gave no adequate nmr spectra.

iv) Hydrogenation over palladium/carbon at 50°C and 5 atmospheres gave very low turnover (<4%) of starting material to a colourless solid. ν_{max} ($CHCl_3$ solution): 3450 (br, N–H), 1710cm^{-1} (s, C=O).

General procedure for Friedel–Craft acylation with oxalyl chloride.

A solution of the respective aromatic compound in DCM (3cm^3 per mmol) was cooled to 0°C, and aluminium chloride added. After stirring for 30 minutes, a solution of oxalyl chloride (1.5 equivalents) in DCM (3cm^3 per mmol) was added dropwise. The reaction was stirred at 0°C for 3 hours, and then poured into an equal volume of ice/water. the mixture was stirred overnight, and the organic phase separated. The aqueous layer was re–extracted, and the combined organic extracts back–extracted with 2M sodium hydroxide. Reacidification yielded a colourless precipitate which was isolated by filtration, or re–extraction.

Conversion to the methyl esters was achieved by disolving the substrate in dry methanol (5cm^3 per mmol), and passing dry HCl gas through the solution, until t.l.c. indicated that the reaction had gone to completion. Removal of the solvent, and extraction of the product between EtOAc and 2M NaOH, yielded the products as liquids.

2–Bromo–4–hydroxybenzoic acid

3–Bromophenol was acylated by the above procedure to yield the title compound as a pale green solid (50%) m.p. 203–207°C (lit.[22] 206–208°C). (Found: C 38.9, 2.3. $C_7H_5BrO_3$ requires: C 38.7, H 2.3). ν_{max} ($CHCl_3$ solution): 3000cm^{-1} (br, –COOH). δ_H (DMSO–d_6): 7.13 (1H, dd, ^3J 8.4, ^4J 2.2Hz, 5–H), 7.22 (1H, d, ^4J 1.8Hz, 3–H), 7.72 (1H, d, ^3J 8.4Hz, 6–H), 11.8–14.0 (1H, br, COOH), 12.2 (1H, br, 4–OH). m/z: 198 (100%, $[M–H_2O]^+$), 200 (100), 216 (41, M^+), 218 (41), 170

(36, $[M-COOH]^+$), 172 (36).

Methyl 2–bromo–4–hydroxybenzoate

The title compound was prepared from 2–bromo–4–hydroxybenzoic acid by the standard method described above in 30 hours, as a colourless liquid (58%). ν_{max} (Liquid film): 3200 (br, –OH), 1680cm–1 (s, C=O). δ_H ($CDCl_3$): 3.95 (3H, s, COOMe), 7.02 (1H, dd, ^3J 8.4, ^4J 1.8Hz, 5–H), 7.18 (1H, d, ^4J 1.8Hz, 3–H), 7.68 (1H, d, ^3J 8.4Hz, 6–H), 10.8 (1H, br, 4–OH). m/z: 198 (100%, $[M-OMe-H]^+$), 200 (100), 230 (45, M^+), 232 (45), 170 (39, $[M-COOMe-H]^+$), 172 (39), 199 (25, $[M-OMe]^+$), 201 (25).

Acylation and methylation of *m*–bromoanisole

A sample of *m*–bromoanisole was reacted in the standard conditions to give a mixture of acids by t.l.c. (31%). Methylation of the mixture gave a mixture of esters which were separable by column chromatography, and shown to consist of methyl 2–bromo–4–hydroxybenzoate (20%), and methyl 2–bromo–4–methoxybenzoate (62%) as a colourless liquid. (High Res. Acc. Mass found: 243.9767. $C_9H_9Br(79)O_3$ requires 243.9733). ν_{max} (liquid film): 2945, 1720 (s, C=O). δ_H ($CDCl_3$): 3.82 (3H, s, $ArOCH_3$), 3.89 (3H, s, $-COOCH_3$), 6.85 (1H, dd, ^3J 8.8, ^4J 2.6Hz, 5–H), 7.18 (1H, d, ^4J 2.6Hz, 3–H), 7.85 (1H, d, ^3J 8.8Hz, 6–H). m/z: 213 (100%, $[M-OMe]^+$), 215 (100), 244 (38, M^+), 246 (38), 170 (10, $[M-COOMe-Me]^+$), 172 (10).

Methyl Benzoylformate (167)

A solution of phenacyl bromide (525mg, 2.64mmol) in methanol ($10cm^3$), was heated to reflux, and selenium dioxide (0.35g, 1.1 eq.), added in one portion.

Reflux was continued for 18 hours, whereupon the reaction was allowed to cool. The solution (containing black coloured colloidal selenium metal), was diluted with water, and extracted into ether. The solid obtained on removal of solvent *in vacuo*, was purified by column chromatography to yield the title compound as a colourless oil (282mg, 65%). ν_{max} (liquid film): 1730, 1685cm^{-1} (2xC=O). δ_H ($CDCl_3$): 3.07 (3H, s, O-Me), 6.60 (2H, tm, 3,5-H), 6.75 (1H, tt, 4-H), 7.1 (2H, dm, 2,6-H). m/z (low eV E.I.): 105 (100%, [M-COOMe]$^+$), 165 (37, M$^+$), 106 (8).

2-(2,4-Dimethoxyphenyl)ethanedione, dimethylacetal (181)

A solution of 2-bromo-2',4'-dimethoxyacetophenone (150mg, 0.58mmol) in dry methanol (10cm^3) was heated at reflux, and selenium dioxide (100mg, 1.5equivalent) added in one portion. The reaction was heated at reflux overnight, cooled, and diluted with water. The product was extracted into diethylether, and dried ($MgSO_4$). The major spot by t.l.c. [R_F (30% ethyl acetate/petrol) 0.3] was isolated by column chromatography, giving the title compound as a colourless oil (60mg, 43%). ν_{max}: 1670 (s), 1600 cm^{-1} (s). δ_H ($CDCl_3$): 3.42 (6H, s, -OMe), 3.85 (3H, s, Ar-OMe), 3.90 (3H, s, Ar-OMe), 5.48 (1H, s, Ar-CO-C*H*), 6.45 (1H, d, ^4J 2.4Hz, 3-H), 6.53 (1H, dd, ^3J 8.8, ^4J 2.4Hz 5-H), 7.79 (1H, d, ^3J 8.8Hz, 6-H). δ_C ($CDCl_3$): 54.0 (q, -OMe), 55.7 and 55.4 (q, Ar-OMe), 98.4 (d, C-3), 102.5 (d, Ar-CO-C), 105.4 (d, C-5), 119.2 (s, C-1), 133.1 (d, C-6), 160.7 (s, C-2), 164.7 (s, C-4), 193.7 (s, Ar-*C*O-C). m/z: 75 (100%), 165 (44, [M-C(OMe)$_2$]$^+$), 181 (9), [240 (2%, M$^+$)].

4-Methyl-3-nitroanisole (187)

4-Methyl-3-nitrophenol (10.03g, 65.5mmol) was added to a solution of potassium

carbonate (14g), in acetone (300cm^3). After all the organic material had dissolved, iodomethane (7cm^3) was added to the dark red coloured solution. The reaction was stirred at room temperature, until t.l.c. indicated that all the starting material had been consumed (about 26hrs.). The solution was filtered through celite, and the acetone and excess iodomethane removed *in vacuo*. The residue was partitioned between ethyl acetate and water. The organic extracts were washed with water, and dried ($MgSO_4$). Removal of solvent *in vacuo* yielded a gum which solidified on cooling in a cardice/acetone bath to give the product as a yellow solid (10.9g, 99%), m.p. 17°C (lit.[23] 17°C).

6–Methoxyindole (189)

i) with *2,2-bis-(6-methoxyindol-3-yl)propane* (190) as by–product.

A mixture of 4–methyl–3–nitroanisole (1.06g, 6.32mmol) and tripiperidinomethane (2.5g, 1.5 equivalents) as heated to 100°C under reduced pressure due to a water pump. Heating was continued until all the starting material had been consumed (by t.l.c. about 3 hours). The reaction was cooled to room temperature, and then washed (with the aid of a minimum amount of acetone) into a separaring funnel containing 30% aqueous titanium chloride (20cm^3, 6 equivalents) and 4M ammonium acetate (50cm^3). The separating funnel was stoppered, and shaken vigorously for 20 minutes, and then the organic material copiously extracted into ether. The ether extracts were combined, and dried ($MgSO_4$). T.l.c. of the solution revealed two major products which gave very coloured spots on treatment of the plate with iron (III) chloride/perchloric acid (indicating that the products were indoles). Evaporation of solvent *in vacuo* and column chromatography yielded 6–methoxyindole [R_F (30%EtOAc/petrol[60–80°C]) 0.5] as a colourless solid (0.6g, 66%) m.p. 90°C (lit.[24] 91–2°C). ν_{max} ($CHCl_3$

solution): 3480cm^{-1} (s, –NH). δ_H ($CDCl_3$): 3.79 (3H, s, 6–OCH_3), 6.45 (1H, m, 3–H), 6.78 (2H, m, 5,7–H), 7.00 (1H, dd, ^3J 2.3, 3.3Hz, 2–H), 7.50 (1H, d, ^4J 8.6Hz, 4–H), 7.9 (1H, br, N–H). m/z: 147 (100%, M^+), 132 (85, $[M–Me]^+$), 104 (30).

Also isolated was *2,2-bis-(6-methoxyindol-3-yl)propane* [R_F (30% EtOAc/petrol) 0.3] as a colourless solid (0.15mg, 15%) m.p. 212–3°C. (Found: C 76.1, H 6.7, N 8.1. Calc for $C_{21}N_{22}N_2O_2$: C 75.4, H 6.65, N 8.4%). ν_{max} (nujol): 3450cm^{-1} (s, –NH). δ_H (DMSO–d_6): 1.76 (6H, s, 1,3–Me), 3.66 (6H, s, 6'–OMe), 6.35 (2H, dd, ^3J 8.8, ^4J 2.2Hz, 5'–H), 6.77 (2H, d, ^4J 2.2Hz, 7'–H), 6.99 (2H, d, ^3J 8.8Hz, 4'–H), 7.09 (2H, d, ^3J 2.2Hz, 2'–H). δ_C (DMSO–d_6): 30.0 (q, C–1,3), 33.9 (s, C–2), 54.8 (q, 6'–OMe), 94.2, 107.6, 119.1, 120.6 (d, Arom. C–H), 120.3, 123.7, 137.6, 154.7 (s, quaternary). m/z: 160 (100%, [M–indole–2Me]$^+$), 161 (53), 319 (12, $[M–Me]^+$), 334 (6, M^+), 335 (2).

ii) Using THF as co–solvent

The reaction procedure detailed above for the preparation of 6–methoxyindole was repeated using THF to transfer the styrene into the separating funnel rather than acetone. This yielded 6–methoxyindole in 75% yield.

1–Ethyl–2,3–dihydro–6–methoxyindole (191)

Sodium borohydride (180mg, 48mmol) was added portionwise over a period of 30 minutes to a solution of 6–methoxyindole (72mg, 4.9mmol) in glacial acetic acid (5cm^3). The reaction was stirred overnight, during which time two compounds were noted by t.l.c. The more polar of these finally disappeared, and the reaction was worked up by pouring the solution into water, and stirring for 30 minutes. The mixture was made basic by the addition of 2M sodium hydroxide, and the

suspension extracted into ether. The ether fractions were washed once with water and dried (Na_2SO_4). Evaporation of the ether yielded the title compound as an oil (73mg, 85%). ν_{max} (liquid film): 1610cm^{-1}. δ_H ($CDCl_3$): 1.16 (3H, t, ^3J 7.2Hz, N–CH_2–CH_3), 2.87 (2H, t, ^3J 8.1Hz, 2–H), 3.10 (2H, q, ^3J 7.2Hz, N–CH_2–CH_3), 3.33 (2H, t, ^3J 8.2Hz, 3–H), 3.75 (3H, s, 6–OMe), 6.06 (1H, d, ^4J 2.1Hz, 7–H), 6.16 (1H, dd, ^3J 7.9, ^4J 2.4Hz, 5–H), 6.93 (1H, d, ^3J 7.9Hz, 4–H). m/z: 162 (100%, $[M–Me]^+$), 177 (46, M^+), 178 (24), 163 (14).

2,3–dihydro–6–methoxyindole (186)

Sodium cyanoborohydride (126mg, 2mmol) was added portionwise to a solution of 6–methoxyindole (60mg, 4mmol) in glacial acetic acid (5cm^3). The reaction was stirred at room temperature for 3 hours, poured into water, stirred for a further 30 minutes, and neutralised with sodium hydroxide. The product was isolated by extraction into ether, washing the organic extracts, and drying (Na_2SO_4). Evaporation of the solvent *in vacuo* and column chromatography yielded the title compound [R_F (30% EtOAc/petrol [60–80°C] 0.5] as a colourless oil (39mg, 65%). ν_{max} (liquid film): 3470cm^{-1} (br, N–H). δ_H ($CDCl_3$): 2.95 (2H, t(d), ^3J 8.25Hz, 2–H), 3.54 (2H, t, ^3J 8.25Hz, 3–H), 3.6 (1H, br, N–H), 3.74 (3H, s, 6–OMe), 6.25 (2H, m, 4,7–H), 6.98 (1H, dd, 5–H). m/z: 149 (100%, M^+), 148 (90), 117 (17, $[M–H–OMe]^+$), 133 (16, $[M–H–Me]^+$), 150 (10).

The hydrochloride salt was prepared, by passing HCl gas through a solution of the indoline in ether. The precipated salt was collected by filtration as a colourless solid, m.p. 219°C (Lit.[25] 222–224°C). (Found: C 58.4, H 6.5, N 7.3. Calc. for $C_9H_{12}NOCl$: C 58.2, H 6.5, N 7.3%).

1–Acetyl–2,3–dihydro–6–methoxyindole (192)

2,3-dihydro-6-methoxyindole (560mg, 3.75mmol) was dissolved and stirred for 5 minutes in acetic anhydride (15cm^3). The solution was poured into water, and vigorously stirred for 1 hour. The product was collected by filtration to yield the title compound as a colourless solid (390mg, 66%) m.p. 99-100°C [from DCM/petrol(60-80°C)] (lit.[26] 105°C). (Found: C 68.8, H 6.9, N 7.2. Calc. for $C_{11}H_{13}NO_2$: C 69.1, H 6.85, N 7.2%). ν_{max} 1720cm$_{-1}$ (s, -C=O). δ_H ($CDCl_3$): 2.15 (3H, s, $COCH_3$), 3.04 (2H, t(br), ^3J 8.4Hz, 2-H), 3.77 (3H, s, 6-OMe), 3.97 (2H, t, ^3J 8.5Hz, 3-H), 6.53 (1H, dd, ^3J 8.2, ^4J 2.5Hz, 5-H), 7.00 (1H, d, ^3J 8.1Hz, 4-H), 7.88 (1H, d, ^4J 2.6Hz, 7-H). m/z: 149 (100%, $[M-Ac+H]^+$), 148 (73), 191 (64, M^+), 43 (20, Ac^+), 177 (15, $[M-Me+1]^+$).

2-Bromo-1-(1-acetyl-2,3-dihydro-6-methoxyindol-5-yl)ethanone (193)

A flame dried flask was charged under an inert atmosphere with a solution of 1-acetyl-2,3-dihydro-6-methoxyindole (52mg, 2.7mmol) in DCM (5cm^3). The solution was cooled to 0°C, and aluminium chloride (100mg, 3 equivalents) added in one portion. The reaction was stirred for 1 hour, and bromoacetyl chloride (0.02cm^3, 2.7mmol) added. The reaction was stirred for 6 hours, and 2M hydrochloric acid added (15cm^3). The two phase solution was stirred vigorously for 20 minutes, and the organic layer diluted with more DCM. The organic layer was separated, and the acidic layer extracted with more DCM. The combined DCM extracts were washed once with water, and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a colourless solid. This was passed through a short column of silica to remove a small amount of polar impurities [eluting with 50% EtOAc/petrol (60-80°C)] to yield the title compound as a colourless solid (60mg, 71%). (Found: C 49.7, H 4.41, N 4.57. $C_{13}H_{14}BrNO_3$ requires: C 50.0, H 4.5, N 4.5%). δ_H ($CDCl_3$): 2.26 (3H, s, NCO-Me), 3.15 (2H, t, ^3J 8.4Hz, 2-H), 3.95 (3H, s, 6-OMe), 4.12 (2H, t, ^3J 8.5Hz, 3-H), 4.60 (2H, s, $COCH_2Br$), 7.70 (1H, s,

7-H), 7.97 (1H, s, 4-H). m/z: 218 (100%, $[M-Ac-Br]^+$), 176 (69, $[M-Ac-CH_2Br]^+$), 43 (31), 204 (26, $[M-Ac-CO]^+$), 162 (22, $[M-Ac-CO-Br]^+$), 311 (20, M^+), 313 (19).

2-(1-acetyl-2,3-dihydro-6-methoxyindol-5-yl)ethanedione, 1-dimethylacetal (194)

A solution of 2-bromo-(1-acetyl-2,3-dihydro-6-methoxyindol-5-yl)ethanone (60mg, 0.29mmol) in methanol ($5cm^3$) was heated to reflux, and selenium dioxide (25mg, 1.1 equivalents) added in one portion. The solution was refluxed overnight, cooled, and diluted with water ($25cm^3$). The organic material was extracted into ether, which was dried ($MgSO_4$). Column chromatography yielded the title compound [R_f (50% EtOAc/petrol[60-80°C]) 0.1] as a colourless gum (18mg, 32%), and starting material (20mg, 33%). ν_{max} (liquid film): $1610cm^{-1}$. m/z (*iso*-butyl C.I.): 89 (100%), 218 (94, $[M-(MeO)_2CH]^+$), 234 (92), 262 (61, $[M-OMe]^+$), 294 (52, M^+), 264 (28).

Synthesis of *p*-methylbenzenesulphonyl protected 6-hydroxyindoles.

4-Methyl-3-nitrophenol, 4-methylbenzenesulphonyl ester

The procedure for the synthesis of 4-methyl-3-nitroanisole was repeated using *p*-toluenesulphonyl choride as the electrophilic agent, to yield the title compound as a colourless solid (83%) m.p. 109-110°C (from EtOH/water) (lit. 91°C). ν_{max} (nujol): $1530cm^{-1}$. δ_H ($CDCl_3$): 2.3 (3H, s, Me), 2.4 (3H, s, Me), 7.7-7.9 (7H, m). m/z: 91 (100%, $[C_7H_7]^+$), 155 (71, $[C_7H_7SO_2]^+$), 65 (14, $[C_6H_5]^+$), 307 (13, M^+).

Indol-6-ol, 4-methylbenzenesulphonyl ester

The procedure for the synthesis of 6-methoxyindole was repeated using 4-methylbenzenesulphonate ester of 4-methyl-3-nitrophenol as starting material, and THF as co-solvent, to yield the title compound as a colourless solid (66%), m.p. 111°C (from ethanol/water). (Found: C 63.4, H 4.6, N 4.8. Calc. for $C_{15}H_{13}NO_3S$: C 62.7, H 4.6, N 4.85%). ν_{max} (nujol): 3440cm^{-1} (br, N-H). δ_H ($CDCl_3$): 2.39 (3H, s, 4'-Me), 6.47 (1H, m, 3-H), 6.60 (1H, dd, ^3J 8.6, ^4J 2.2Hz, 5-H), 7.15 (1H, d(m), ^4J 2.2Hz, 7-H), 7.17 (1H, dd, ^3J 2.4, 3.1Hz, 2-H), 7.24 (2H, d(m), ^3J 7.9Hz, 2',6'-H), 7.43 (1H, d, ^3J 8.4Hz, 4-H), 7.68 (2H, d(m), ^3J 3',5'-H), 8.42 (1H, br, N-H). m/z: 132 (100%, [M-tosyl]$^+$), 287 (18, M$^+$), 133 (11).

2,3-Dihydroindol-6-ol, 4-methylbenzenesulphonate ester

The tosyl protected hydroxyindole was reduced with sodium cyanoborohydride in glacial acetic acid as described for 2,3-dihydro-6-methoxyindole to yield the title compound as a colourless solid (86%) m.p. 80°C. (Found: C 62.2, H 5.2, N 4.8. Calc. for $C_{15}H_{15}NO_3S$: C 62.3, H 5.2, N 4.85%). ν_{max} (Nujol): 3390cm^{-1} (s, N-H). δ_H ($CDCl_3$): 2.42 (3H, s, 4'-Me), 2.94 (2H, t(d), ^3J 8.3Hz, 2-H), 3.16 (1H, br, N-H), 3.54 (2H, t, ^3J 8.5Hz, 3-H), 6.15 (1H, dd, ^3J 8.1, ^4J 2.2Hz, 5-H), 6.28 (1H, d, ^4J 2.2Hz, 7-H), 6.89 (1H, d, ^3J 7.9Hz, 4-H), 7.28 (2H, d(m), ^3J 8.1Hz, 2',6'-H), 7.70 (2H, d, ^3J 8.2Hz, 3',5'-H). m/z (low eV E.I.): 289 (100%, M$^+$), 290 (17), 225 (16), 291 (6).

1-Acetyl-2,3-dihydroindol-6-ol, 4-methylbenzensulphonyl ester

The *O*-tosyl protected hydroxyindoline was acetylated with acetic anhydride as described for the methoxyindoline, to yield the title compound as a colourless solid (95%) m.p. 155°C. (Found: C 61.7, H 5.1, N 4.3. Calc. for $C_{17}H_{17}NO_4S$: C

61.6, H 5.15, N 4.3%). ν_{max} (nujol): 1665cm−1. δ_H ($CDCl_3$): 2.13 (3H, s, NCOMe), 2.43 (3H, s, 1'–Me), 3.11 (2H, t, ^3J 8.5Hz, 3–H), 4.01 (2H, t, ^3J 8.5Hz, 2–H), 6.66 (1H, dd, ^3J 8.1, ^4J 2.2Hz, 5–H), 7.02 (1H, d, ^3J 8.2Hz, 4–H), 7.31 (2H, d, ^3J 8.25Hz, 2',6'–H), 7.72 (2H, d, ^3J 8.25Hz, 3',5'–H), 7.82 (1H, d, ^4J 2.0Hz, 7–H). m/z: 289 (100%, $[M-Ac+1]^+$), 331 (84, M^+), 106 (65), 91 (49, $[C_7H_7]^+$), 43 (47, Ac^+), 134 (30, [M–Ac–tosyl]$^+$), 225 (29).

References to chapter 6

1. G. H. Svodoba and D. A. Black, *Phytochemistry and Pharmacology of Catharanthus Roseus,* , p. 45Dekker, New York (1975).
2. R. L. Margolis and L. Wilson, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 2466.
3. R. C. Skoda , R. Janssi , and P. Christen , *Biochem. Biophys. Res. Commun.*, 1983, **115**, 144.
4. P. Magnus, *Lecture Delivered at Autumn Congress of R. S. C.*, 1986, University of Bath.
5. D. Raileanu, O. Constantinescu-Simon, E. Monescu, and C. Nenitzescu, *Rev. Roum. Chim.*, 1967, **12**, 105.
6. R. J. Sundberg , R. Broome , C. P. Walters, and D. Schnur, *J. Heterocyclic Chem.* , 1981, **18**, 807.
7. A. R. Katritsky and K. Akutagawa, *Tetrahedron Letts.*, 1985, **26**, 5935.
8. A. Closse, W. Haefliger, and D. Hauser, *J. Med. Chem.*, 1981, **24**, 1465.
9. E. J. Corey and J. P. Schaefer, *J. Am. Chem. Soc.*, 1960, **82**, 918.
10. K. B. Sharpless and K. M. Gordon, *J. Am. Chem. Soc.*, 1976, **98**, 300.
11. U. Pinder and R. Adam, *J. Heterocyclic Chem.*, 1988, **25**, 1.
12. D. H. Lloyd and D. E. Nichols, *Tetrahedron Letts.*, 1983, **24**, 4561.
13. R. A. Swaringen, J. F. Eaddy, and T. R. Henderson, *J. Org. Chem.*, 1980, **45**, 3986.
14. K. K. Vong, *PhD Thesis, University of Bath*, 1987.
15. G. W. Gribble, P. D. Lord, J. Skotnicki, S. E. Dietz, J. T. Eaton, and J. L. Johnson, *J. Am. Chem. Soc.*, 1974, **96**, 7812.
16. Y. Kumar and L. Florvall, *Synth. Commun.*, 1983, **13**, 489.

17. P. Magnus, M. Ladlow, and J. Elliott, *J. Am. Chem. Soc.*, 1987, **109**, 7929.

18. J. G. Jackson and J. Kenner, *J. Chem. Soc.*, 1928, 573.

19. D. Vorlander, *Chem. Ber.*, 1902, **35**, 1683.

20. D. Vorlander and B. Drescher, *Chem. Ber.*, 1901, **34**, 1854.

21. R. J. Sundberg and H. F. Russell, *J. Org. Chem.*, 1973, **38**, 3324.

22. K. Freidrich and H. Oster, *Chem. Ber.*, 1961, **94**, 834.

23. L. Limpach, *Chem. Ber.*, 1891, **24**, 4139.

24. Kermack, Perkin, and Robinson, *J. Chem. Soc.*, 1921, **119**, 1612.

25. H. Liad, S. Aoyagi, and C. Kibayashi, *J. Chem. Soc. Perkin Trans. I*, 1955, **23**, 2502.

26. T. Wieland and O. Ungar, *Chem. Ber.*, 1963, **96**, 253.